Nanomicelle-enhanced, asymmetric ERED-catalyzed reductions of activated olefins. Applications to 1-pot chemo- and bio-catalysis sequences in water

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Electronic Supplementary Information

Table of Contents

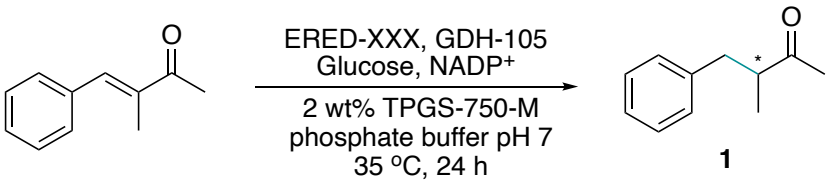
General Information

Silica gel TLC plates (UV 254 indicator, thickness 200 mm standard grade, glass backed and 230-400 mesh from Merck) or Aluminum Oxide 60 F254 polyester basked plates (Sigma-Aldrich, 0.2 mm thick) were used. The developed TLC plate was analyzed by a UV lamp (254 nm). The plates were further analyzed with the use of an aqueous potassium permanganate stain or butanolic vanillin and developed with a heat gun. All commercially available reagents were used without further purification. A 2 wt % TPGS-750-M/H_2O solution was prepared by dissolving TPGS-750-M in degassed HPLC grade water. TPGS-750-M[1] was made as described previously and is also commercially available. Reagents were purchased from Sigma-Aldrich, Combi-Blocks, Alfa Aesar, or Acros Organics. Flash chromatography was performed using Silicycle Silicaflash® P60 unbonded grade silica. Codex® Ene Reductase Screening kit is commercially available from Codexis. The ^{1}H and ^{13}C NMR were recorded at 25 ºC on either a Varian Unity Inova 500 MHz or a Varian Unity Inova 600 MHz spectrometers in $CDCl_3$ with residual $CHCl_3$ (^{1}H = 7.26 ppm, ^{13}C = 77.16 ppm) as the internal standard. Chemical shifts are reported in parts per million (ppm). The data presented will be reported as follows; chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, quin = quintet, m = multiplet), coupling constant (if applicable) and integration. HRMS data were recorded on a Waters Micromass LCT TOF ES+ Premier mass spectrometer using ESI ionization. Chiral HPLC data were collected using an Agilent 1220 HPLC. HRMS data were recorded on a Waters Micromass. LCT α-values were measured on a Perkin Elmer Polarimeter 341 in a cuvette (l=10cm) at 589 nm (Na lamp). Concentration *c* is given in g/100mL.

Preparation of Buffer Solution

Aqueous 1 M stock solutions of potassium phosphate monobasic (**A**) and potassium phosphate dibasic (**B**) were prepared. A pH 7 phosphate buffer solution was then prepared by mixing 38.5 mL of solution **A** with 61.5 mL of solution **B**. The pH was controlled and adjusted, if needed, with a 1 M solution of NaOH or HCl. The buffer solution was diluted with HPLC grade water to 0.1 M. 2 wt % of TPGS-750-M, as a wax, was dissolved and used as media of the reaction. 4 and 6 wt % of TPGS-750-M in the buffer solution have also been prepared. TPGS-750-M is available from Sigma-Aldrich (catalog #733857 (solution) or #763896 (wax)). Potassium phosphate monobasic and dibasic were purchased from Sigma Aldrich.

Enzyme Screen - Table S1



1

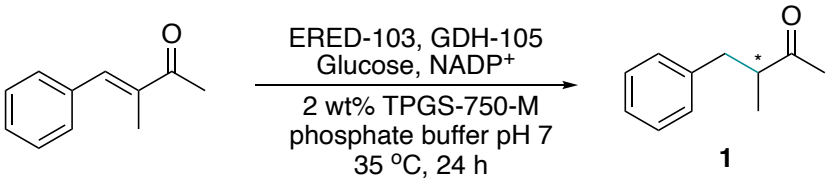
Entry	Enzyme	Conversion (%)	ee (%)
1	ERED-103	99	86
2	ERED-110	97	83
3	ERED-112	99	77
4	ERED-207	92	20
5	ERED-P1-A04	93	88
6	ERED-P1-E01	91	90
7	ERED-P1-H09	65	34

To evaluate the impact of TGPS-750-M on the conversion of the model substrate by ENE-reductase, comparative monitoring was performed. In a 5-dr vial, GHD-105 (20 mg), Glucose (2 equiv, 123 mg) and $NADP^+$ (5 mg) were added followed by addition of 10 mL of phosphate buffer at pH 7 (with or without 2 wt % of TPGS-750-M) to make a stock solution. The vial was lightly swirled to allow the components to fully dissolve. Enone (5 mg) and ENE-reductases (10 mg) were added into seven labeled 1-dr equipped with magnetic stir bars. The stock solution (containing GDH-105, glucose, and $NADP^+$ in phosphate buffer, 1 mL) was added to each 1-dr vial. The reactions were stirred at 35 °C for 24 h. After 24 h, the reactions were extracted with MTBE (5x) and concentrated *in vacuo.* The samples were analyzed by ^1H NMR to determine the conversion. (2.47 ppm (s) → 1.11 ppm (d)). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/*i*-propanol 99.5:0.5, flow rate 0.5 mL/min) t_1 15.2 min (minor) t_2 16.42 min (major).

Conversion Monitoring in Phosphate Buffer and TPGS-750-M/Phosphate Buffer

To evaluate the impact of TGPS-750-M on the conversion of four different substrates by ENE-reductase, comparative monitoring with and without surfactant in buffer was performed. In a 5-dr vial, GHD-105 (20 mg), Glucose (2 equiv relative to substrate) and $NADP^+$ (5 mg) were added followed by addition of 10 mL of phosphate buffer at pH = 7 (with or without 2 wt % of TPGS-750-M) to make a stock solution (for substrate **4**, 4 and 6 wt % of TPGS-750-M in the buffer solution were also prepared). The vial was lightly swirled to allow the components to fully dissolve. In 1-dr vials equipped with a magnetic stir bar, enone (5 mg) and ERED-103 (10 mg) were added. The stock solution (containing GDH-105, glucose, and $NADP^+$ in phosphate buffer, 1 mL) was added to each 1-dr vial. The reactions were stirred at 35 °C. At each time interval noted, the reactions were extracted with MTBE (5x) and concentrated *in vacuo* and monitored by ^1H NMR.

Concentration Effect Studies – Table S2



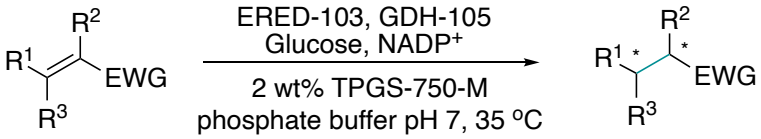
1

Entry	Concentration (M)	Conversion (%)
1	0.03	99
2	0.05	99
3	0.10	80
4	0.50	24

To evaluate the impact of increased concentration on the activity of the enzyme in 2 wt % of TPGS-750-M in phosphate buffer, reaction monitoring was performed. In a 1-dr vial equipped with a magnetic stir bar, GHD-105 (2 mg), Glucose (2 equiv to enone, 12.3 mg) and $NADP^+$ (0.5 mg), enone (5 mg) and ERED (10 mg) were added followed by addition of 2 wt % of TPGS-750-M in phosphate buffer at pH = 7 to reach desired concentration (poor stirring of components was observed at 0.5 M). The reactions were stirred at 35 °C for 24 h. After 24 h the organic layers were extracted with MTBE

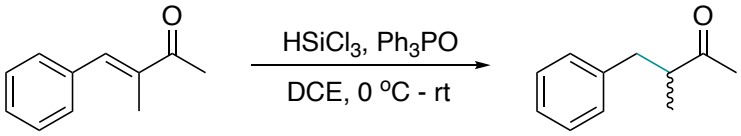
(5x) and concentrated *in vacuo.* The samples were analyzed by ^{1}H NMR to determine the conversion. (2.47 ppm (s) → 1.11 ppm (d)).

General Procedure for Enantioselective Reduction of Activated Olefins



To a 5-dr vial equipped with a magnetic stir bar was added olefin (50 mg), ERED-103 (70 mg), GDH-105 (20 mg), glucose (2 equiv to olefin), and $NADP^+$ (5 mg). 5-7 mL (substrate dependent) of 2 wt % of TPGS-750-M in phosphate buffer at pH = 7 was added to the vial. The reaction was set to stir at 35 °C. The reaction was monitored via TLC or ^{1}H NMR. Upon completion, the organic layers were extracted with MTBE (5x), dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude material was purified via flash chromatography to afford the unsaturated alkane. Absolute stereochemistry was confirmed by comparison to commercially available and / or literature referenced enantiopure compounds. All other products were assigned by analogy.

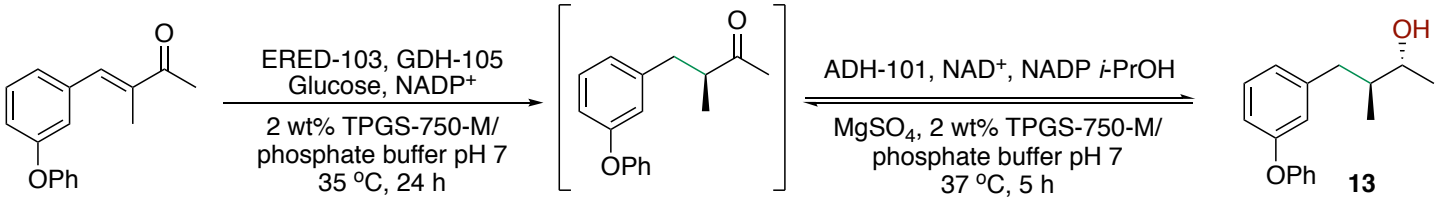
General Procedure for Reduction of Activated Olefins



Racemic ketones were synthesized from a modified literature procedure.[2] To an oven-dried round bottom flask, equipped with a magnetic stir bar was added enone (1.0 equiv) and triphenylphosphine oxide (1.0 equiv) The flask was evacuated and backfilled with argon (3x). DCE [0.25M] was added to the flask and the mixture was cooled to 0 °C. Trichlorosilane (2.0 equiv) was added dropwise into the reaction mixture at 0 °C. The reaction was warmed to rt and set to stir for 3-7 h. The reaction was monitored via TLC. Upon completion, the reaction mixture was quenched with saturated $NaHCO_3$ solution and filtered through Celite. The organic layer was extracted with DCM (3x), dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude material was purified via flash chromatography to afford the unsaturated alkane. The resulting alkane was utilized to determine enantiomeric excess.

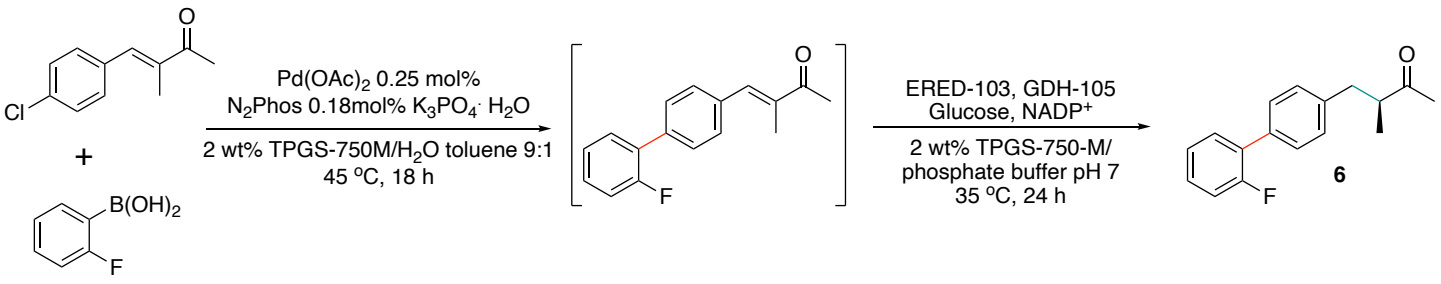
For compounds **11** and **13**, the racemic ketones were synthesized from a modified literature procedure.[3] To a solution of Pd/C (10 wt % 10 mg) in THF (5.0 mL), were added enal or enone (1.50 mmol, 1.0 equiv), H_2SO_4 (100 μL), and triethylsilane (1.1 equiv). The reactions were set to stir for 6 h. Upon completion the mixture was filtered through a pad of alumina with DCM as the eluent. The organic layer was concentrated *in vacuo* and the crude material was purified by flash chromatography.

1-Pot ERED Reduction, then ADH Reduction



ADH-101[4] is commercially available within the enzyme kit EZK-001 from Johnson Matthey. NAD^+ was purchased from Bioworld. Isopropanol was purchased from VWR. All other commercially available reagents were used without further purification. To a 5-dr vial equipped with a magnetic stir bar was added olefin (50 mg), ERED-103 (70 mg), GDH-105 (20 mg), $NADP^+$ (5 mg) and glucose (2 equiv to olefin). 5 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 °C for 24 h. The reaction was monitored via TLC. Upon completion, $MgSO_4$ (0.8 mg), NAD^+ (2.6 mg), $NADP^+$ (2.4 mg) and ADH-101 (20 mg) were added in succession. *i*-PrOH (0.6 mL) was added to the reaction mixture. The reaction was stirred at 35 °C for 5 h. The reaction was extracted with EtOAc (5x). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude material was purified via flash chromatography. See analytical data *(vide infra*), page S15.

1-Pot Pd-catalyzed Suzuki-Miyaura coupling, ERED Reduction

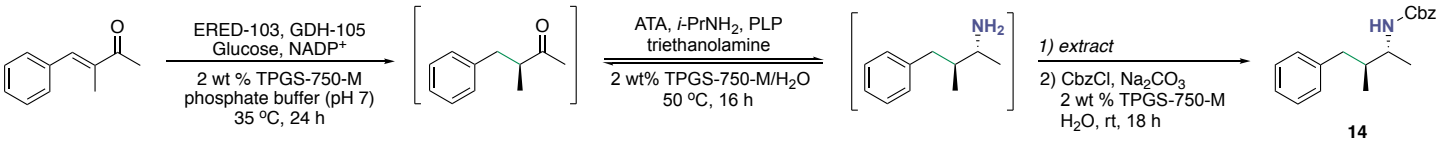


To an oven dried 1-dram vile equipped with a stir bar was added $Pd(OAc)_2$ (2.25 mg, 0.01 mmol) and N_2Phos[5] (14.8 mg, 0.018 mmol). The vial was capped with a 14/20 rubber septum sealed with Teflon tape. The vial was evacuated and backfilled with argon three times and left under a continuous

flow of argon. Anhydrous toluene (1 mL) was added to the vile to achieve the desired Pd concentration (50 μL of stock solution equates to 1000 ppm loading for a 0.5 mmol reaction). The mixture was set to stir for 15 min. At this point the catalyst is ready and may be added to the reaction mixture.

To an oven dried, 10 mL flask equipped with a magnetic stir bar was charged aryl chloride (0.25 mmol), organoboron (0.38 mmol), and potassium phosphate (0.38 mmol). The vial was fitted with a rubber septum and sealed with Teflon tape. The reaction flask was purged with argon with the use of a vent needle. At this point, a solution of 2 wt % TPGS-750-M in (0.9 mL) followed by the catalyst solution (125 μL) via syringe. The reaction was monitored by GC-MS. Upon completion of the reaction, the pH was adjusted to 7 with a 1 M HCl and, ERED-103 (70 mg), GDH-105 (20 mg), $NADP^+$ (5 mg) and glucose (2 equiv to olefin) were added to the flask. 5 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 ºC for 24 h. Upon completion the reaction was filtered over a pad of Celite and the organics were extracted with MTBE (5x). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude material was purified via flash chromatography. See analytical data *(vide infra*), page S12.

1-Pot ERED Reduction, then Amine Formation utilizing Transaminase ATA



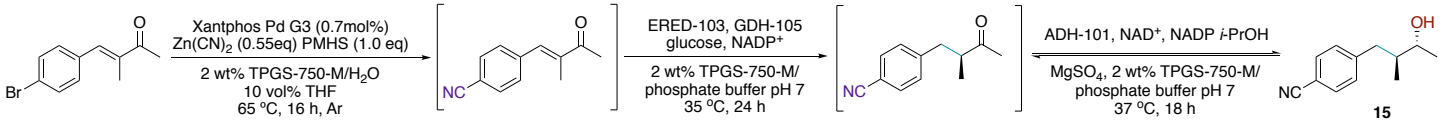
To a 5-dr vial equipped with a magnetic stir bar was added olefin (20 mg, $1.272.10^{-4}$ mol), ERED-103 (40 mg), GDH-105 (8 mg), glucose (46 mg), and $NADP^+$ (2 mg). 4 mL of 2 wt % of TPGS-750-M in phosphate buffer at pH = 7 was added to the vial. The reaction was set to stir at 35 ºC overnight. The reaction was monitored via TLC or ^{1}H NMR. The concentration was adjusted to [0.01 M] by adding 8.72 mL of a fresh solution* (triethanolamine, [118 mM], pH = 8.5) previously made (see below the protocol). The ATA-256 (100 mg) was then added and the reaction was stirred at 50 °C. Upon completion, the reaction was quenched by adding 5 N NaOH (1.5 mL) to increase reaction pH > 12 and extracted into EtOAc (5 x 10 mL). The organic layers separated by centrifugation were combined and evaporated. To a 2-dr vial containing the product from the second step were added 2 wt % of TPGS-750-M in water (0.9 mL) and sodium carbonate (40 mg, 3 equiv). The reaction was cooled to 0°C and benzyl chloroformate (18 µL, 1 equiv) was added. The reaction was stirred for 20 min at 0 °C and allowed to warm to rt and stirred overnight. The solution is acidified to pH = 2 (0 °C) and extracted with EtOAc (3

x 1 mL). The organic layers were combined and dried over anhydrous $MgSO_4$ and concentrated *in vacuo.* The crude material was purified via flash chromatography to afford the desired compound.

*Fresh solution for step 2:

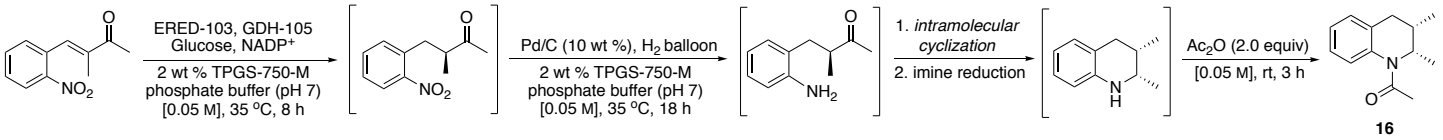
In a 20 mL flask were added triethanolamine (0.21 g), isopropylamine (1.06 mL), PLP (3.4 mg) and 2 wt % of TPGS-750-M in water (4 mL). The pH was adjusted to 8.5 with HCl (12 M). The volume was brought to 8.72 mL by adding 2 wt % of TPGS-750-M in water. See analytical data *(vide infra*), page S16.

1-Pot Pd-catalyzed Cyanation, ERED Reduction, then ADH Reduction



Aryl bromide (1 equiv, 0.2 mmol), $Zn(CN)_2$ (12.9 mg, 0.55 equiv), Xantphos palladacycle (1.7 mg, 0.7 mol %) were added to a 1-dr vial equipped with a magnetic stir bar. The reaction vial was evacuated and backfilled with argon (3x). PMHS (13 μL, 1 equiv) was added under argon followed by dry THF (40 μL, 10 vol %) and 2 wt % TPGS-750-M/H_2O (360 μL). The reaction mixture was stirred at 65°C overnight. The reaction was monitored via TLC. Upon completion, the reaction mixture was transferred to a 5-dr vial containing ERED-103 (70 mg), GDH-105 (20 mg), $NADP^+$ (5 mg) and glucose (2 equiv to olefin). 5.6 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 ºC for 24 h. The reaction was monitored via TLC. Upon completion, $MgSO_4$ (0.8 mg), NAD^+ (2.6 mg), $NADP^+$ (2.4 mg) and ADH-101 (20 mg) were added in succession. *i*-PrOH (0.6 mL) was added to the reaction mixture. The reaction was stirred at 35 °C for 5 h. The reaction was extracted with EtOAc three times. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude material was purified via flash chromatography. See analytical data *(vide infra*), page S16.

1-Pot ERED Reduction, Pd/C Nitro-reduction, then Acylation



To a 5-dr vial equipped with a magnetic stir bar was added olefin (1.0 equiv, 50 mg), ERED-103 (70 mg), GDH-105 (20 mg), $NADP^+$ (5 mg) and glucose (2 equiv to olefin). 4 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 °C for 24 h. The reaction was monitored via TLC. Upon completion, 1 M HCl was added to the reaction until the mixture reached pH 2. 10 wt % Pd/C (20 mg) was then added to the reaction mixture. The reaction vessel was purged with H_2 using a balloon and then fitted with another balloon of H_2. The reaction was allowed to stir at rt overnight, being monitored via TLC. Upon completion, the H_2 balloon was removed and acetic anhydride (2.0 equiv) was added to the mixture. The reaction was set to stir for an additional 4 h, monitored via TLC. Upon completion, the reaction was extracted with EtOAc (5x) and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The crude material was purified via flash chromatography. See analytical data *(vide infra*), page S17.

References

1. Lipshutz, B. H.; Ghorai, S.; Abela, A. R.; Moser, R.; Nishikata, T.; Duplais, C.; Krasovskiy, A.; Gaston, R. D.; Gadwood, R. C. *J. Org. Chem.* **2011**, *76*, 4379–4391.
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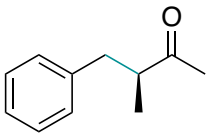
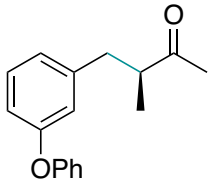
4. (a) Niefind, K.; Müller, J.; Riebel, B.; Hummel, W.; Schomburg, D. *J. Mol. Biol.* **2003,** *327*, 317–328. (b) Schlieben, N. H. *et al. J. Mol. Biol.* **2005,** *349*, 801–813. (c) Leuchs, S.; Greiner, L. *Chem. Biochem. Eng.* **2011***, 25*, 267–281.
5. Akporji, N.; Thakore, R. R.; Cortes-Clerget, M.; Andersen, J.; Landstrom, E.; Aue, D. H.; Gallou, F.; Lipshutz, B. H. *Chem. Sci.* **2020**, *11*, 5205–5212.

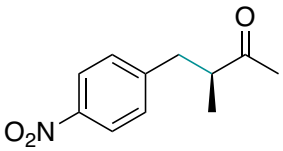
Experimental Data



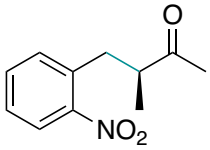
(*S*)-3-Methyl-4-phenylbutan-2-one (1): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 2H), 7.24 – 7.19 (m, 1H), 7.19 – 7.14 (m, 2H), 3.02 (dd, *J* = 13.6, 6.8 Hz, 1H), 2.85 (h, *J* = 7.1 Hz, 1H), 2.58 (dd, *J* = 13.6, 7.7 Hz, 1H), 2.10 (s, 3H), 1.11 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 212.24, 139.80, 129.05, 128.55, 126.37, 48.94, 39.05, 28.98, 16.36. **Yield:** 88% (3 h) as a colorless oil; 86% ee. ***S*** **enantiomer** $\alpha_D^{20.0}$ = +29.8 (c 0.981 in $CHCl_3$) (ERED-103) **R_f** = 0.28 (10% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/*i*-propanol 99.5:0.5, flow rate 0.5 mL/min) t_1 15.2 min (minor) t_2 16.42 min (major).



(*S*)-3-Methyl-4-(3-phenoxyphenyl)butan-2-one (2): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.30 (m, 2H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.4 Hz, 1H), 6.99 (d, *J* = 7.5 Hz, 2H), 6.90 (d, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 9.5 Hz, 2H), 2.98 (dd, *J* = 13.6, 6.7 Hz, 1H), 2.81 (h, *J* = 7.1 Hz, 1H), 2.54 (dd, *J* = 13.6, 7.8 Hz, 1H), 2.10 (s, 3H), 1.09 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ** 212.01, 157.38, 157.34, 141.90, 129.87, 129.81, 124.05, 123.34, 119.51, 118.92, 116.87, 48.73, 38.78, 28.98, 16.39. **Yield**: 91% (24 h) as a pale-yellow oil; 93% ee (ERED-103). **R_f**: 0.25 (10% Et_2O/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/*i*-propanol 98:2, flow rate 1 mL/min) t_1 17.4 min (minor) t_2 18.1 min (major). Molecular formula: $C_{17}H_{18}O_2$ EI-MS $[M\cdot]^+$ calcd: 254.1307; found: 277.1207 $[M + Na]^+$

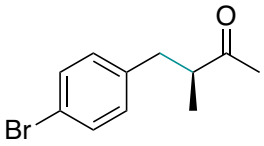


(*S*)-3-Methyl-4-(4-nitrophenyl)-3-butan-2-one (3): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 6.7 Hz, 2H), 3.12 (dd, *J* = 13.6, 7.2 Hz, 1H), 2.85 (p, *J* = 7.1 Hz, 1H), 2.66 (dd, *J* = 13.6, 7.2 Hz, 1H), 2.12 (s, 3H), 1.14 (d, *J* = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ** 210.96, 147.87, 146.75, 129.97, 123.81, 48.50, 38.39, 28.97, 16.72. **Yield**: 87% as a pale-yellow oil; 60% ee. ***S* enantiomer** $\alpha_D^{20.0}$ = +10.1 (c 1.05 in $CHCl_3$) (ERED-103). **R_f**: 0.25 (20% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Lux 5u Cellulose-2 column, hexanes/*i*-propanol 95:5, flow rate 0.5 mL/min) t_1 19.08 min (minor) t_2 20.20 min (major).

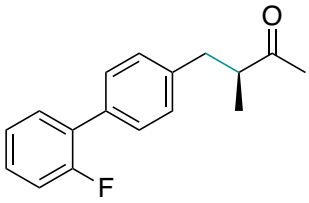


(*S*)-3-Methyl-4-(2-nitrophenyl)butan-2-one (4): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.51 (td, *J* = 7.5, 1.4 Hz, 1H), 7.41 – 7.30 (m, 2H), 3.34 (dd, *J* = 13.3, 6.9 Hz, 1H), 2.97 (h, *J* = 7.0 Hz, 1H), 2.81 (dd, *J* = 13.3, 7.0 Hz, 1H), 2.12 (s, 3H), 1.13 (d, *J* = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ** 211.49, 135.15, 133.40, 133.11, 127.74, 125.17, 47.46, 35.83, 29.13, 16.80. Yield: 87% as a pale-yellow oil; 91% ee (ERED-103). **R_f**: 0.30 (25% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/*i*-propanol 90:10, flow rate 0.7 mL/min) t_1 12.79 min (major) t_2 13.85 min (minor). Spectral data matched those previously reported.[1]

[1] Bogolubsky, A. V. et al. One-Pot Parallel Synthesis Approach to Secondary Amines Based on the Reductive Amination of Ketones. *Synthesis* **2014**, *46*, 1765-1772.

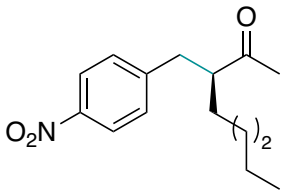


(*S*)-4-(4-Bromophenyl)-3-methyl-3-butan-2-one (5): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 2.95 (dd, *J* = 13.7, 7.0 Hz, 1H), 2.80 (p, *J* = 7.1 Hz, 1H), 2.51 (dd, *J* = 13.7, 7.5 Hz, 1H), 2.09 (s, 3H), 1.09 (dd, *J* = 7.0, 1.3 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 211.74, 138.86, 131.64, 130.8 120.24, 48.78, 38.29, 29.03, 16.47. **Yield:** 86% as a colorless oil; 78% ee (ERED-103). **R_f:** 0.30 (10% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/*i*-propanol 95:5, flow rate 0.5 mL/min) t_1 29.94 min (minor) t_2 31.51 min (major). Spectral data matched those previously reported.[2]

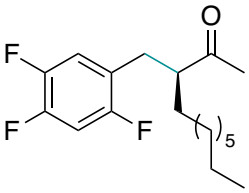


(*S*)-4-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-3-butan-2-one (6): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (m2H), 7.44 (m, 1H), 7.31 (m1H), 7.27 – 7.18 (m, 3H), 7.16 (m, 1H), 3.07 (dd, *J* = 13.6, 6.8 Hz, 1H), 2.89 (h, *J* = 7.0 Hz, 1H), 2.62 (dd, *J* = 13.6, 7.7 Hz, 1H), 2.15 (s, 3H), 1.15 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 212.15, 139.37, 130.79, 130.77, 129.17, 128.99, 128.92, 124.48, 124.45, 116.31, 116.12, 48.89, 38.66, 28.98, 16.48. **Yield**: 82% (73% yield in the 1-pot sequence) as a white solid; 99% ee (ERED-103). **R_f:** 0.30 (10% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/*i*-propanol 98:2, flow rate 1.0 mL/min) t_1 7.23 min (minor) t_2 7.82 min (major). Molecular formula: $C_{17}H_{17}FO$ EI-MS $[M\cdot]^+$ calcd: 256.1263; found: 279.1162 $[M + Na]^+$

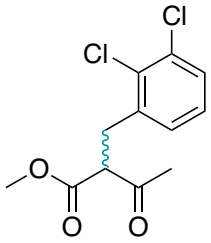
[2] Silva, R. F. et al. Extractive Biocatalysis in the Asymmetric Reduction of α-alkyl, β-aryl enones by Baker's Yeast. *Tetrahedron Asymmetry* **2017**, *28*, 939-944.



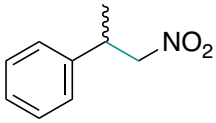
(*S*)-3-(4-Nitrobenzyl)octan-2-one (7): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 2H), 3.02 (dd, *J* = 13.4, 8.6 Hz, 1H), 2.87 – 2.80 (m, 1H), 2.76 (dd, *J* = 13.4, 5.8 Hz, 1H), 2.03 (s, 3H), 1.69 – 1.61 (m, 1H), 1.50 – 1.42 (m, 1H), 1.32 – 1.23 (m, 6H), 0.90 – 0.85 (t, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 211.17, 147.95, 129.87, 123.84, 54.38, 37.17, 31.94, 31.89, 30.22, 26.86, 22.56, 14.10. **Yield**: 84% as a pale-yellow oil; 93% ee (ERED-103). **R_f**: 0.20 (15% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/*i*-propanol 90:10, flow rate 1 mL/min) t_1 7.29 min (major) t_2 8.13 min (minor). Molecular formula: $C_{15}H_{21}NO_3$ EI-MS $[M^{\cdot}]^+$ calcd: 263.1521; found: 263.1523.



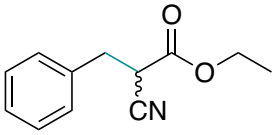
(*S*)-3-(2,4,5-Trifluorobenzyl)undecan-2-one (8): ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.95 (ddd, *J* = 10.5, 8.8, 6.9 Hz, 1H), 6.86 (td, *J* = 9.7, 6.6 Hz, 1H), 2.83 – 2.75 (m, 2H), 2.69 – 2.64 (m, 1H), 2.05 (s, 3H), 1.64 – 1.57 (m, 1H), 1.45 – 1.37 (m, 1H), 1.30 – 1.20 (m, 13H), 0.86 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 211.32, 119.05, 118.95, 105.69, 105.46, 105.29, 53.01, 31.96, 31.77, 30.14, 29.92, 29.77, 29.48, 29.33, 27.13, 22.78, 14.22. **Yield:** 65% as a yellow oil; 98% ee (ERED-103). **R_f**: 0.30 (5% Et_2O/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/*i*-propanol 98:2, flow rate 1 mL/min) t_1 3.55 min (minor) t_2 3.81 min (major). Molecular formula: $C_{18}H_{25}F_3O$ EI-MS $[M^{\cdot}]^+$ calcd: 314.1858; found: 326.1232 $[M + Na]^+$



Methyl 2-(2,3-dichlorobenzyl)-3-oxobutanoate (9): As a 1:1 mixture with the enol ether. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.35 (m, 1H), 7.19 – 7.16 (m, 1H), 7.12 (m, 1H), 3.97 (dd, *J* = 8.2, 6.5 Hz, 1H), 3.71 (s, 3H), 3.35 (dd, *J* = 14.0, 6.6 Hz, 1H), 3.28 (dd, *J* = 14.0, 8.2 Hz, 1H), 2.25 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 201.90, 169.30, 129.94, 129.35, 128.92, 128.68, 127.42, 58.45, 42.99, 32.83, 28.77. **Yield:** 92% as a colorless oil (ERED-103). **R_f:** 0.25 (15% EtOAc/hexanes). Molecular formula: $C_{12}H_{12}Cl_2O_3$ EI-MS $[M\cdot]^+$ calcd: 274.0163; found: 275.0241 $[M + H]^+$



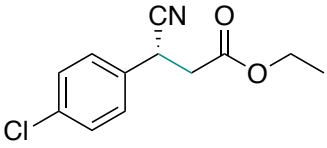
(1-Nitropropan-2-yl)benzene (10): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (t, *J* = 7.3 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 1H), 7.15 (d, *J* = 7.1 Hz, 2H), 4.77 (h, *J* = 6.8 Hz, 1H), 3.32 (dd, *J* = 14.0, 7.4 Hz, 1H), 3.00 (dd, *J* = 14.0, 6.9 Hz, 1H), 1.53 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 135.64, 129.10, 128.94, 127.54, 84.55, 41.29, 18.92. **Yield:** 91% as a colorless oil (ERED-103) **R_f**: 0.27 (5% EtOAc/hexanes). Spectral data matched those previously reported.[3]



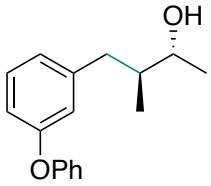
Ethyl 2-cyano-3-phenyl-2-propanoate (11): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 8.0, 6.4 Hz, 2H), 7.34 – 7.27 (m, 3H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.73 (dd, *J* = 8.4, 5.8 Hz, 1H), 3.30 (dd, *J* = 13.8, 5.8 Hz, 1H), 3.21 (dd, *J* = 13.8, 8.4 Hz, 1H), 1.28 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 165.64, 135.41, 129.15, 128.99, 127.91, 116.27, 63.05, 39.80, 35.89, 14.05. **Yield**: 96% as a pale-yellow oil (ERED-P1-A04). **R_f:** 0.30 (10% EtOAc/hexanes). Spectral data matched those previously reported.[4]

[3] Hostmann, T. et al. Light-Enabled Enantiodivergence: Stereospecific Reduction of Activated Alkenes Using a Single Organocatalyst Enantiomer. *Org. Lett.* **2019**, *21*, 10164-10168

[4] Jain, K.; Das, K. A Convenient Method for the Synthesis of Fluoronated α-cyanoacetates via phase-transfer catalysis. *Syn. Comm.* **2018**, *48,* 1966-1973.

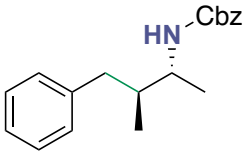


Ethyl (*R*)-3-(4-chlorophenyl)-3-cyanopropanoate (12): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 4.29 (t, *J* = 7.4 Hz, 1H), 4.19 (qq, *J* = 7.1, 3.7 Hz, 2H), 3.01 (dd, *J* = 16.6, 7.8 Hz, 1H), 2.83 (dd, *J* = 16.6, 7.0 Hz, 1H), 1.26 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 169.03, 134.84, 133.11, 129.62, 128.94, 119.64, 61.74, 40.00, 32.76, 14.22. Yield: 82% as a colorless oil, ***R* enantiomer** $\alpha_D^{20.0}$ = -8.1 (c0.991 in $CHCl_3$) 82% ee (ERED-P1-H09). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/*i*-propanol 98:2, flow rate 1.0 mL/min) t_1 17.75 min (major) t_2 19.92 min (minor). Spectral data matched those previously reported.[5]

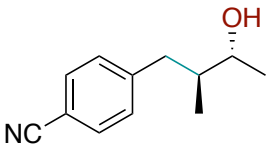


(2*R*,3*S*)-3-Methyl-4-(3-phenoxyphenyl)butan-2-ol (13): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 8.6, 7.4 Hz, 2H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.04 – 6.99 (m, 2H), 6.94 (dt, *J* = 7.7, 1.3 Hz, 1H), 6.88 – 6.83 (m, 2H), 3.70 (p, *J* = 6.2 Hz, 1H), 2.86 (d, *J* = 13.4, 4.9 Hz, 1H), 2.34 (dd, *J* = 13.4, 9.4 Hz, 1H), 1.86 – 1.75 (m, 1H), 1.49 (s, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 157.23, 143.32, 129.84, 129.59, 124.35, 123.19, 119.91, 118.84, 116.52, 71.51, 42.27, 39.07, 20.00, 14.78. **Yield**: 82% as a colorless oil; 77% ee, 56% de (ERED-103, ADH-101). The enantioselectivity was determined by HPLC analysis (Lux 5u Cellulose-2 column, hexanes/*i*-propanol 99:1, flow rate 0.5 mL/min) t_1 16.94 min (minor) t_2 19.93 min (major). Molecular formula: $C_{17}H_{18}O_2$ EI-MS $[M\cdot]^+$ calcd: 254.1307; found: 236.1210 $[M - H_2O]^+$

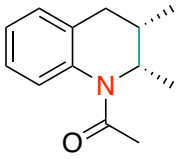
[5] Thakur, V. V.; Nikalje, M. D. Enantioselective Synthesis of R-(-)-baclofen via Ru(II)-BINAP Catalyzed Asymmetric Hydrogenation. *Tetrahedron Asymmetry*, **2003**, *14*, 581-586.



Benzyl ((2*R*,3*S*)-3-methyl-4-phenylbutan-2-yl)carbamate (14): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.29 (m, 5H), 7.29 – 7.24, 7.23 (m, 2H) – 7.04 (m, 3H), 5.11 (s, 2H), 4.61 (s, 1H), 3.83 (s, 1H), 2.76 (dd, *J* = 13.4, 5.4 Hz, 1H), 2.32 (dd, *J* = 13.4, 9.1 Hz, 1H), 1.89 (s, 1H), 1.15 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 155.9, 140.8, 136.7, 129.1 (2C), 128.6, (2C) 128.3 (3C), 128.2 (2C), 125.9, 66.6, 50.5, 40.3, 39.6, 18.4, 14.5. **Yield**: 63% as a yellow oil; 79% ee; 78% de (ERED-103, ATA-256. The enantioselectivity was determined by HPLC analysis (Chiracel AD-H column, hexanes/*i*-propanol 98:2, flow rate 0.7 mL/min) t_1 43.14 min (major) t_2 55.52 min (minor) **HRMS (m/z):** $[M+Na]^+$ calcd. for $C_{19}H_{23}NO_2Na$, 320.1627; found, 320.1629

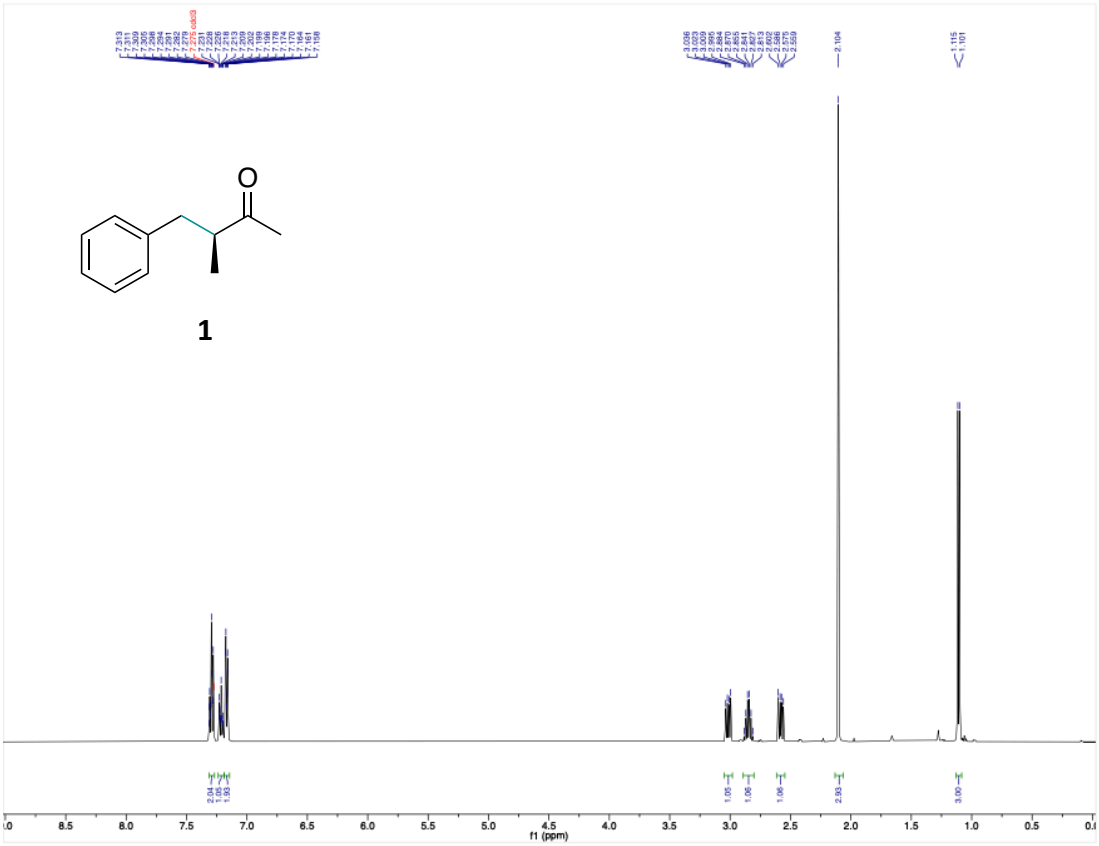


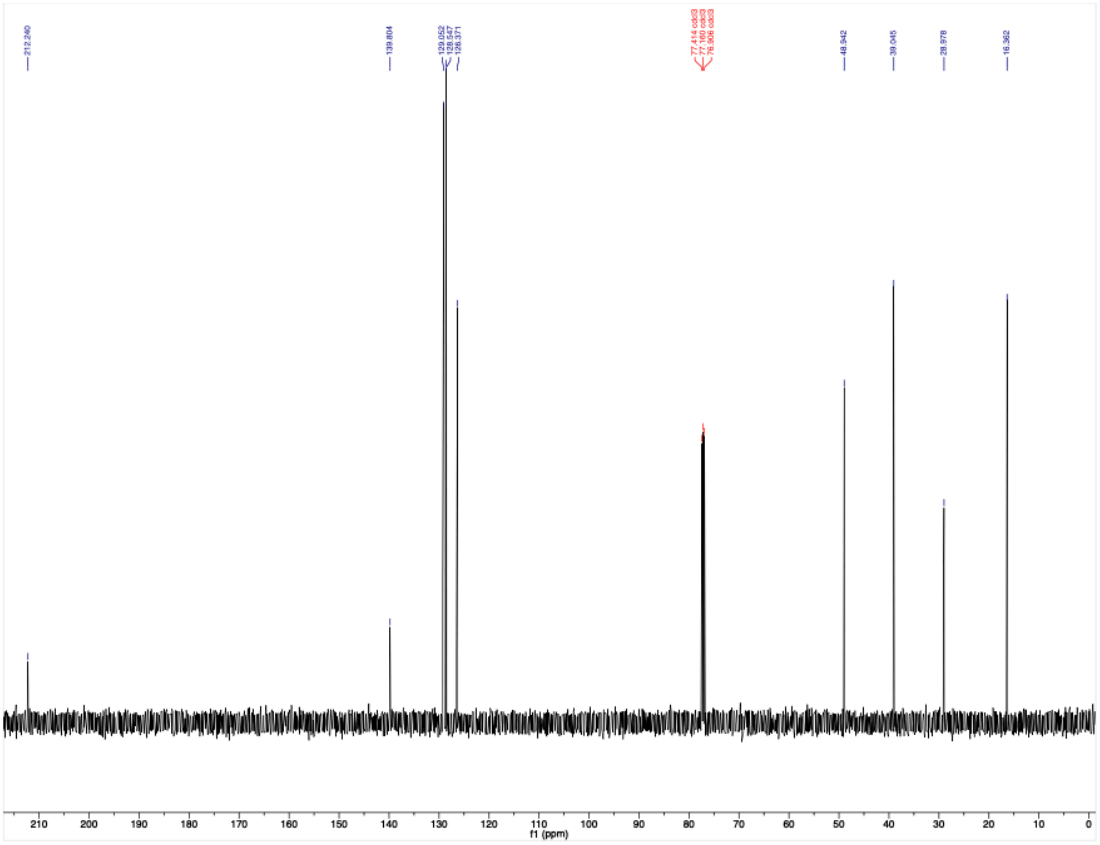
4-((2*S*,3*R*)-3-Hydroxy-2-methylbutyl)benzonitrile (15) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 7.9 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 3.65 (p, *J* = 6.2 Hz, 1H), 2.98 (dd, *J* = 13.4, 4.5 Hz, 1H), 2.39 (dd, *J* = 13.4, 9.6 Hz, 1H), 1.78 (dtd, *J* = 12.4, 6.5, 3.3 Hz, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 0.79 (d, *J* = 6.8 Hz, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ** 147.15, 132.19, 132.15, 130.10, 130.05, 119.24, 109.75, 71.33, 42.17, 39.17, 20.45, 14.89. **Yield:** 54% as a light-yellow oil; >99% ee; 12:88 dr (ERED-103, ADH-101). **R_f** 0.31 (35% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/*i*-propanol 98:2, flow rate 0.7 mL/min) t_1 52.31 min (minor) t_2 54.02 min (major). Molecular formula: $C_{12}H_{15}NO$ EI-MS $[M^{\cdot}]^+$ calcd: 189.1154; found: 171.1053 $[M - H_2O]^+$

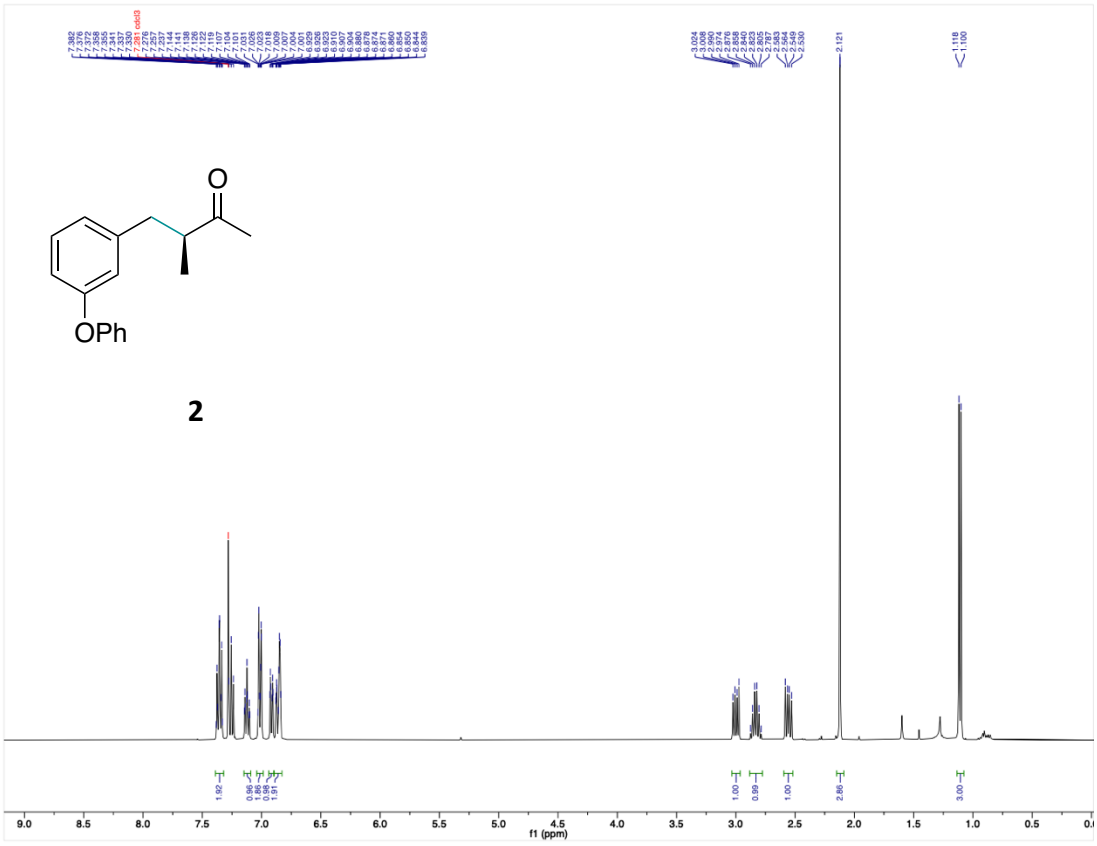


1-((2*S*,3*S*)-2,3-Dimethyl-3,4-dihydroquinolin-1(2*H*)-yl)ethan-1-one (16): *syn : anti* = 90:10; For *syn* isomer: **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.13 (m, 4H), 2.92 (dd, *J* = 17.5, 6.7 Hz, 1H), 2.44 (dd, *J* = 17.5, 11.6 Hz, 1H), 2.24 (s, 3H), 2.21 – 2.15 (m, 1H), 1.14 (t, *J* = 6.9 Hz, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 170.08, 129.19, 125.86, 125.61, 124.88, 32.37, 32.07, 29.85, 23.75, 19.75, 18.76. **Yield:** 62% as a yellow oil; >99% ee; 72:28 dr (ERED-103). **R_f:** 0.30 (25% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/*i*-propanol 95:5, flow rate 0.8 mL/min) t_1 7.04 min (minor) t_2 8.34 min (major). Molecular formula: $C_{13}H_{17}NO$ EI-MS $[M\cdot]^+$ calcd: 203.1310; found: 204.1388 $[M + H]^+$

^{1}H, ^{13}C Spectra of Products







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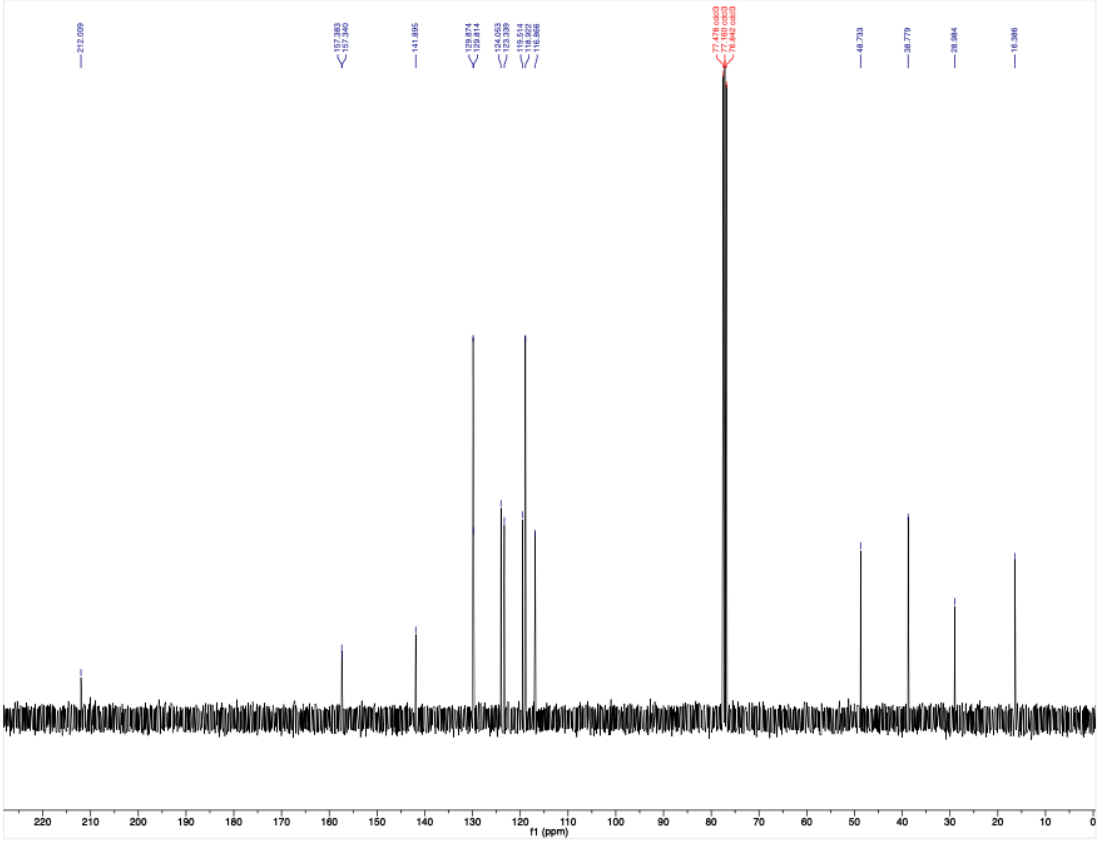


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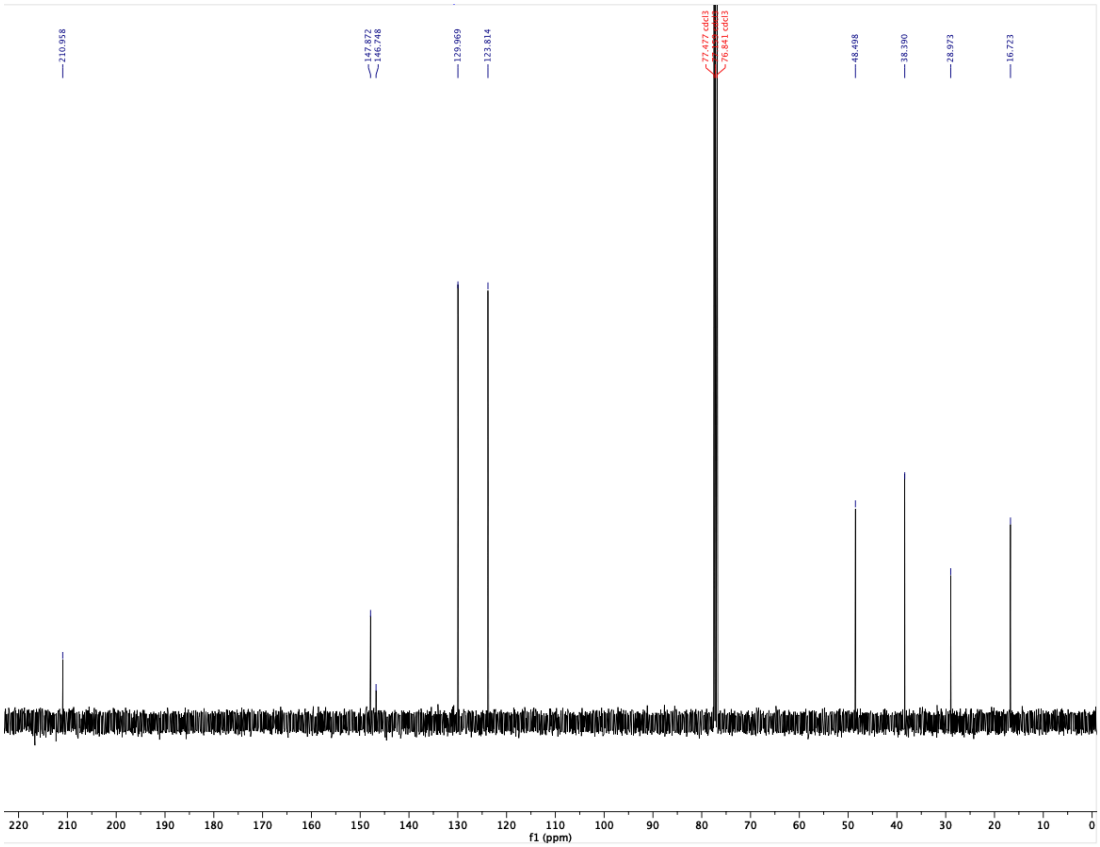
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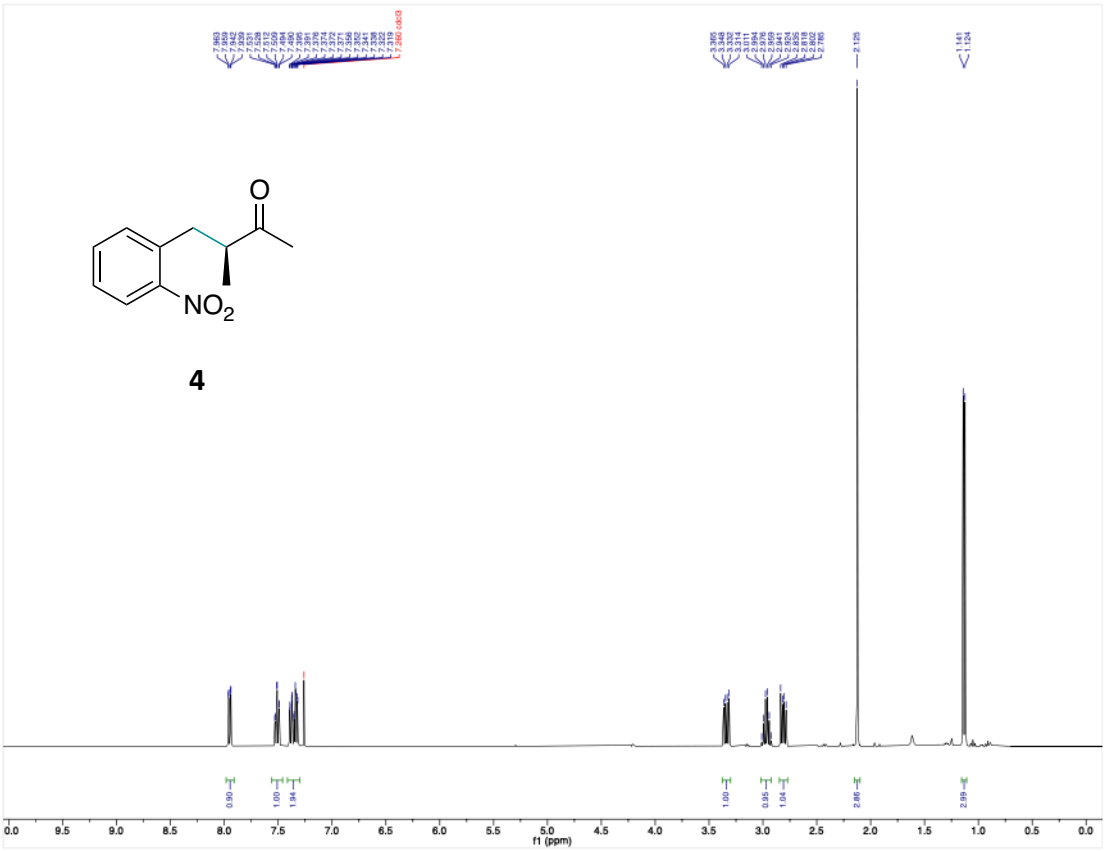

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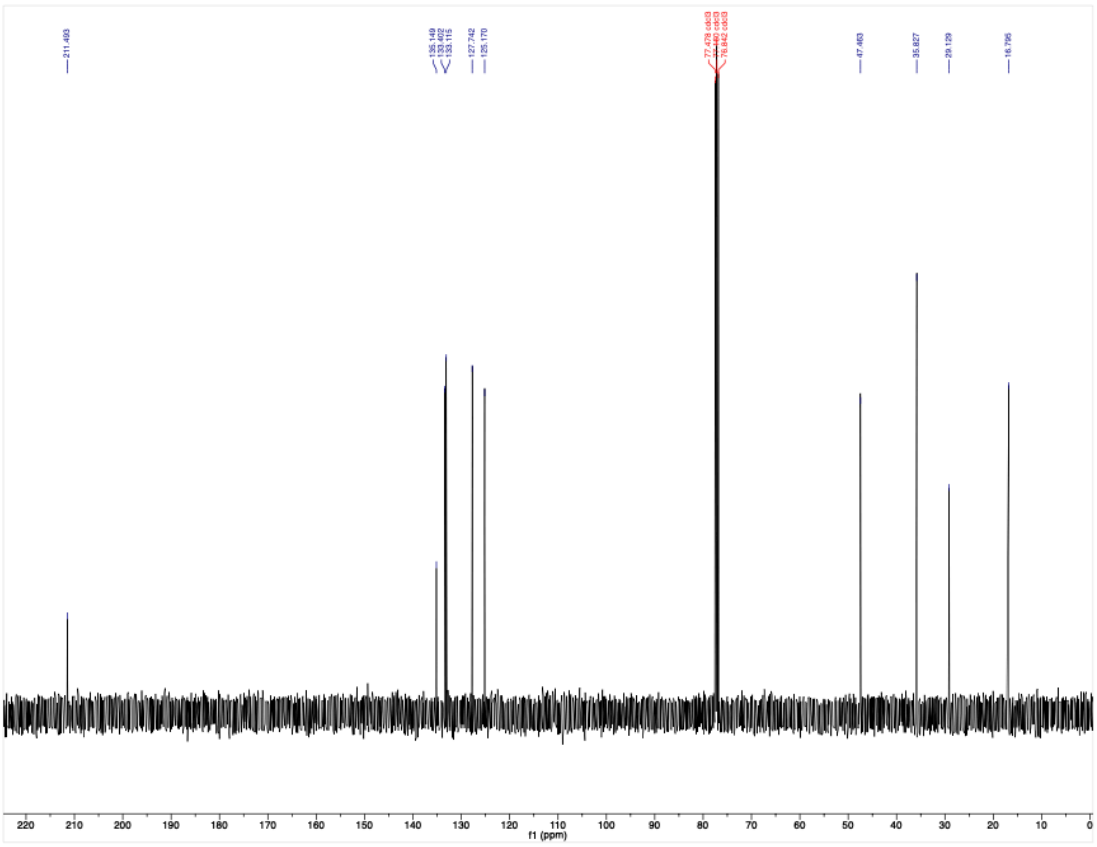

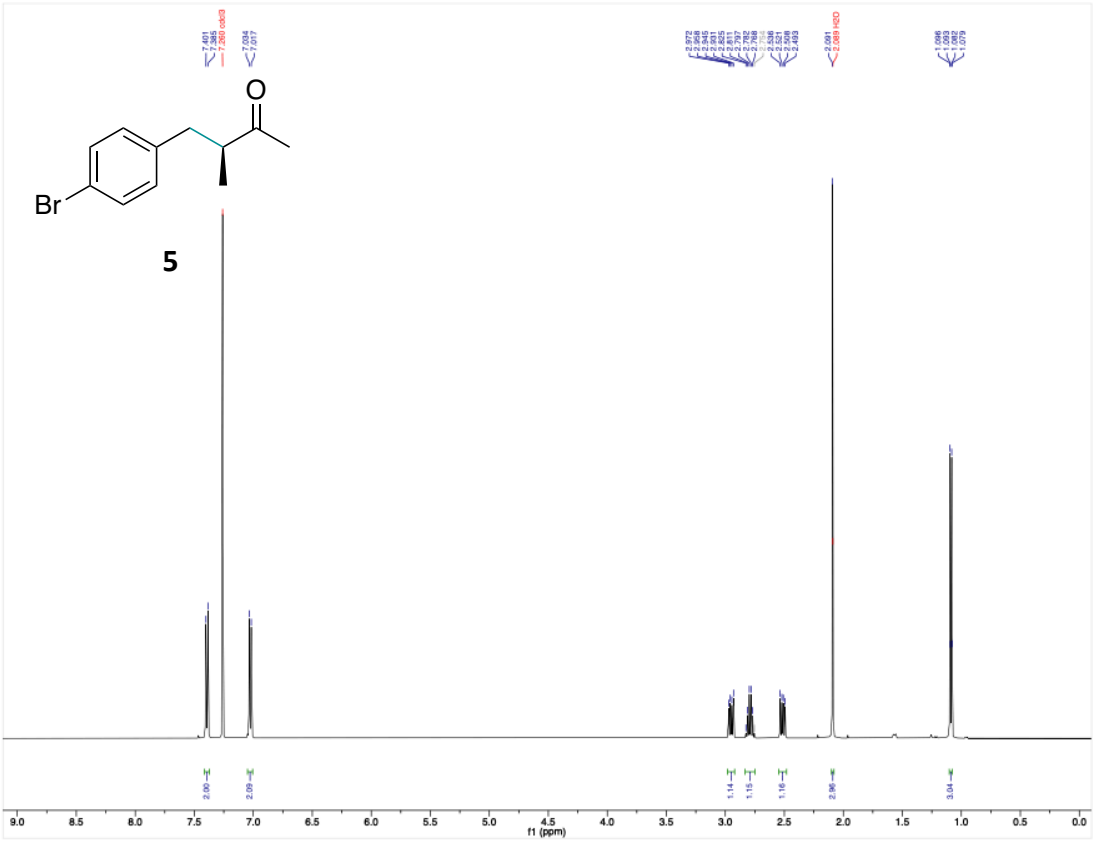
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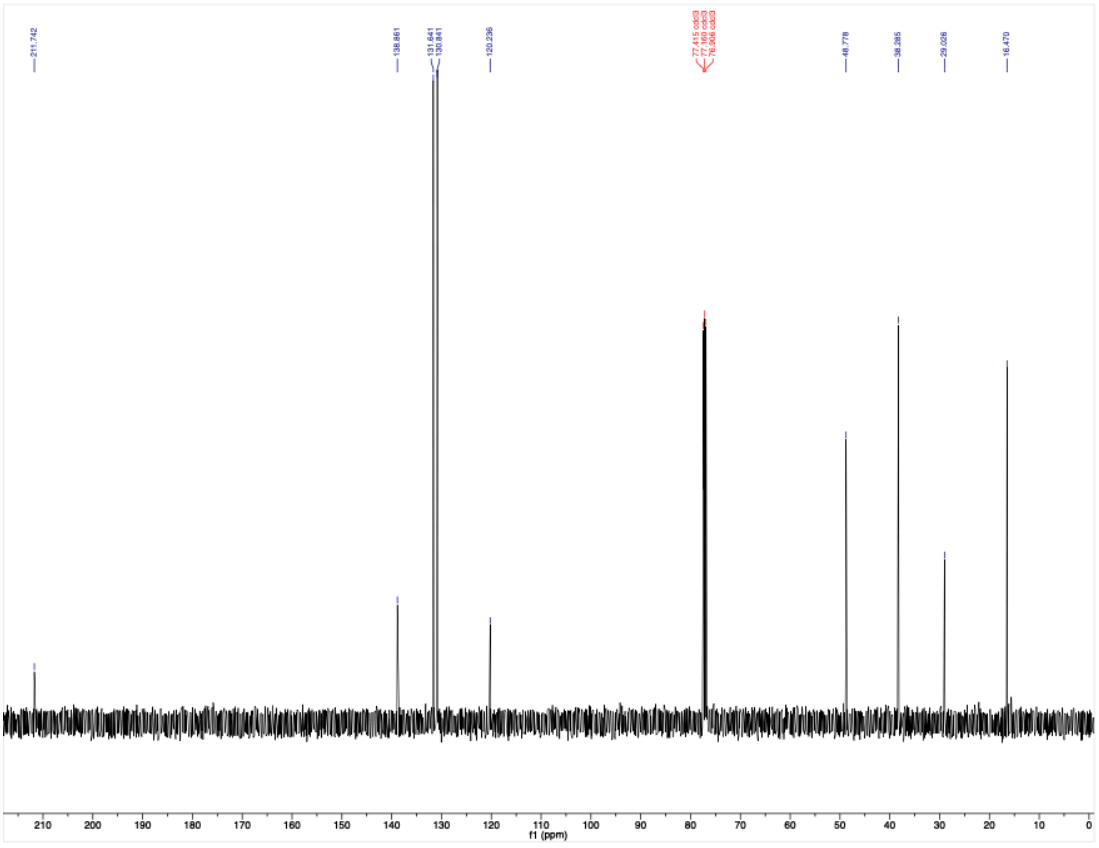
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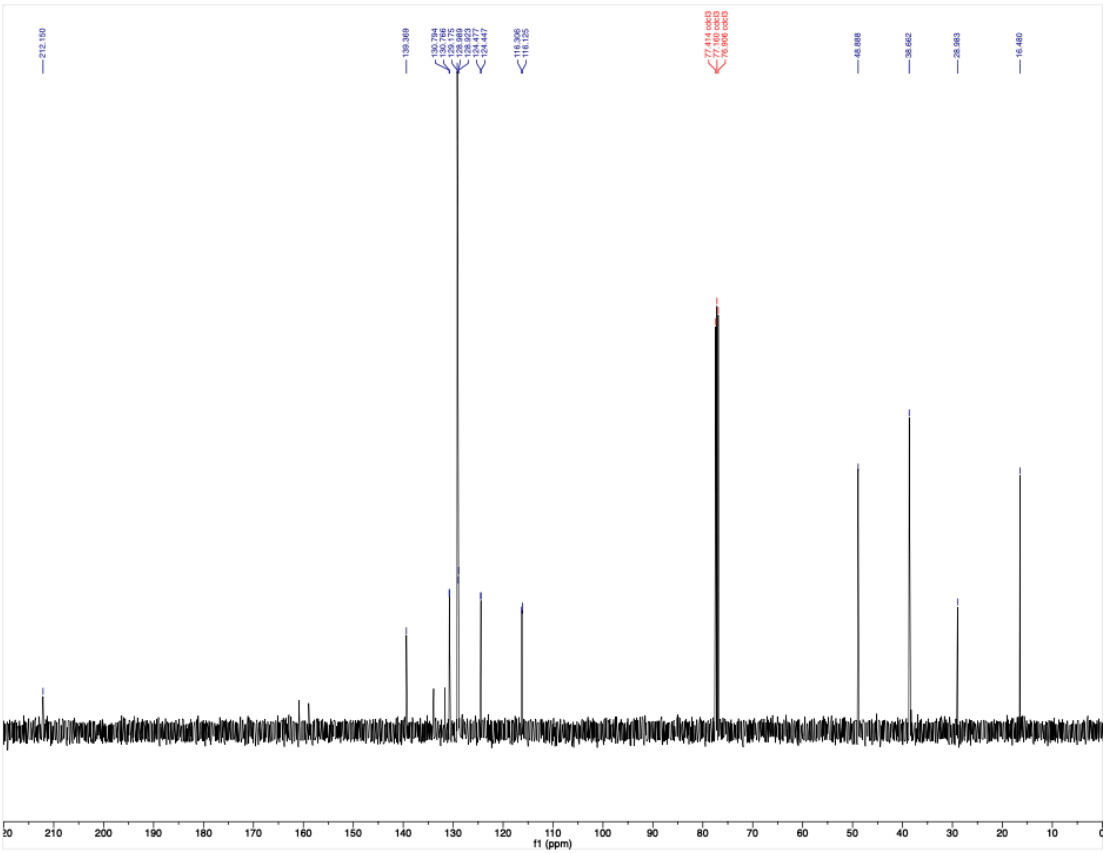
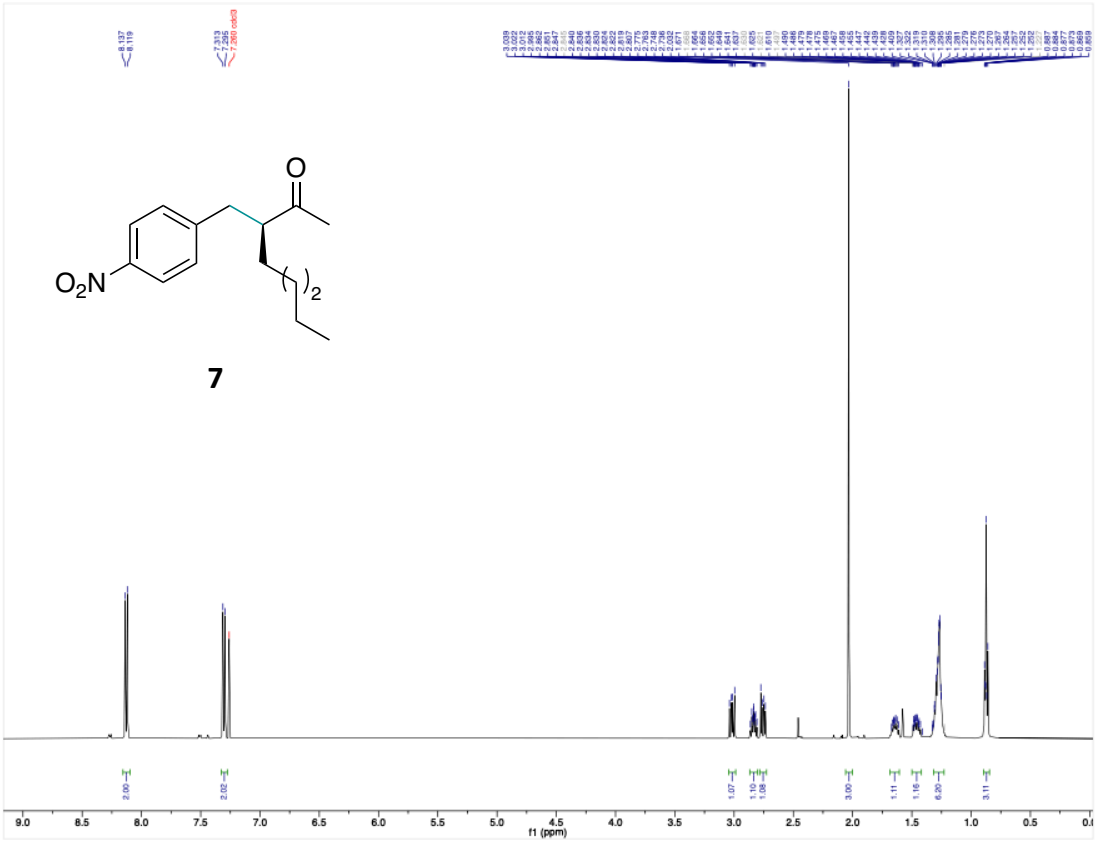
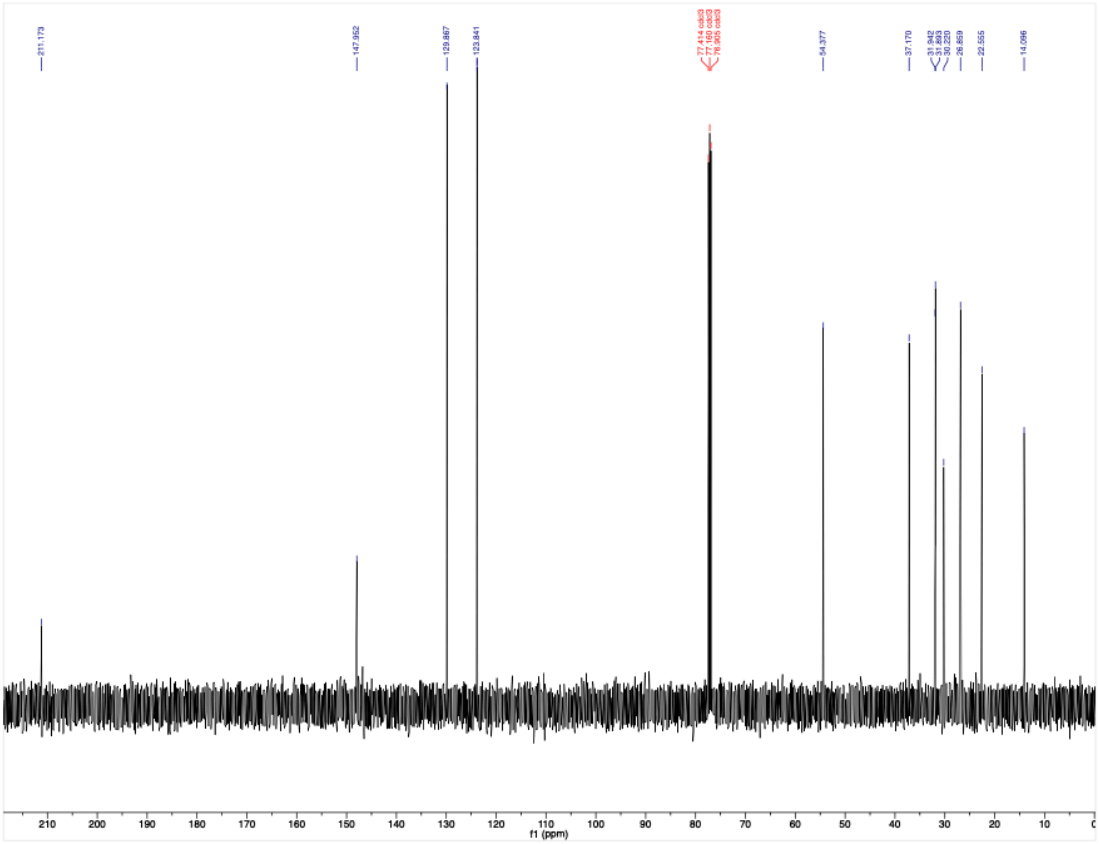
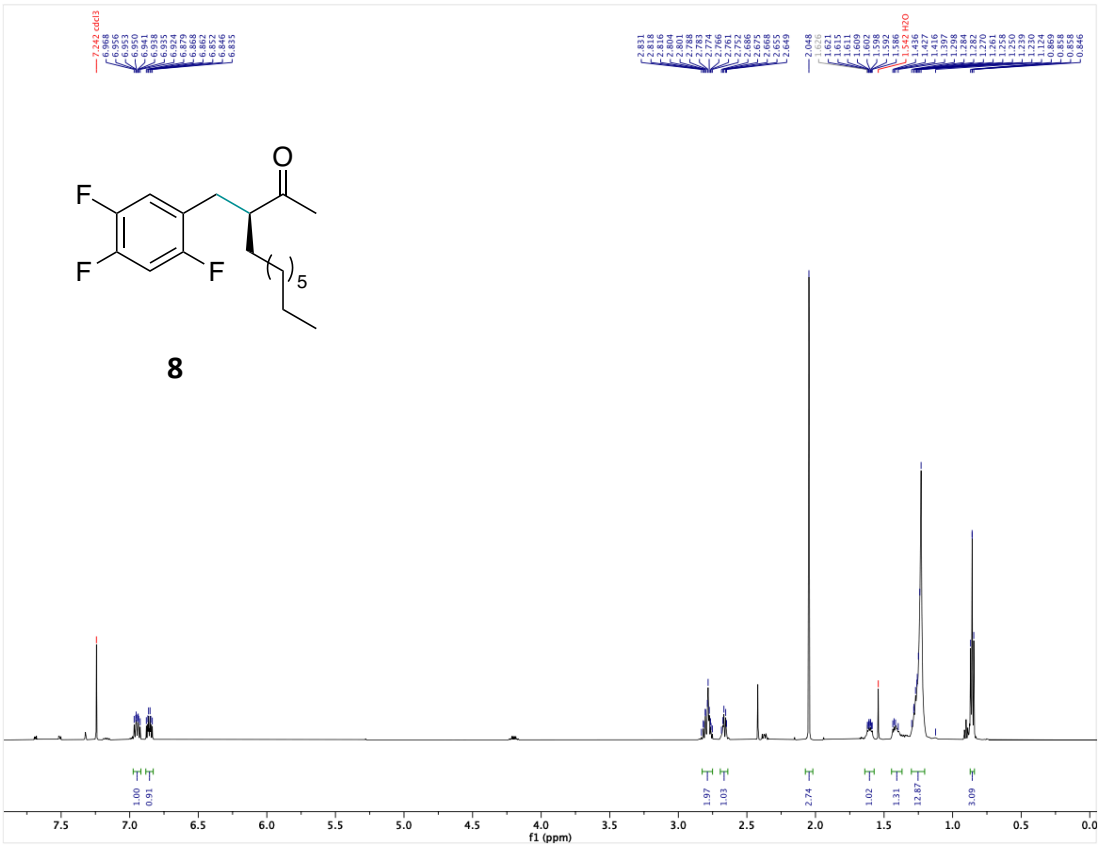
6
f1 (ppm)


212.150
139.369
130.794
130.766
128.975
128.889
126.923
124.477
124.447
116.306
116.125
77.414
77.000
76.586
48.888
38.662
28.983
16.480
f1 (ppm)

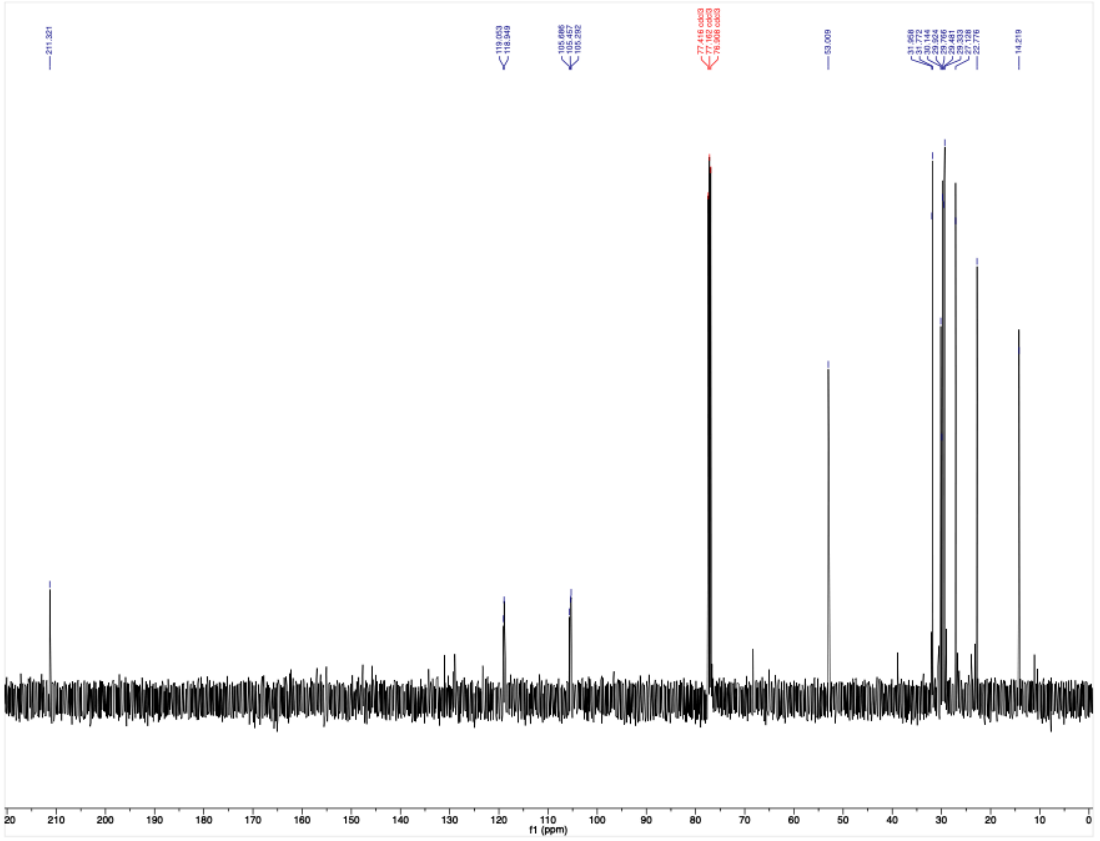


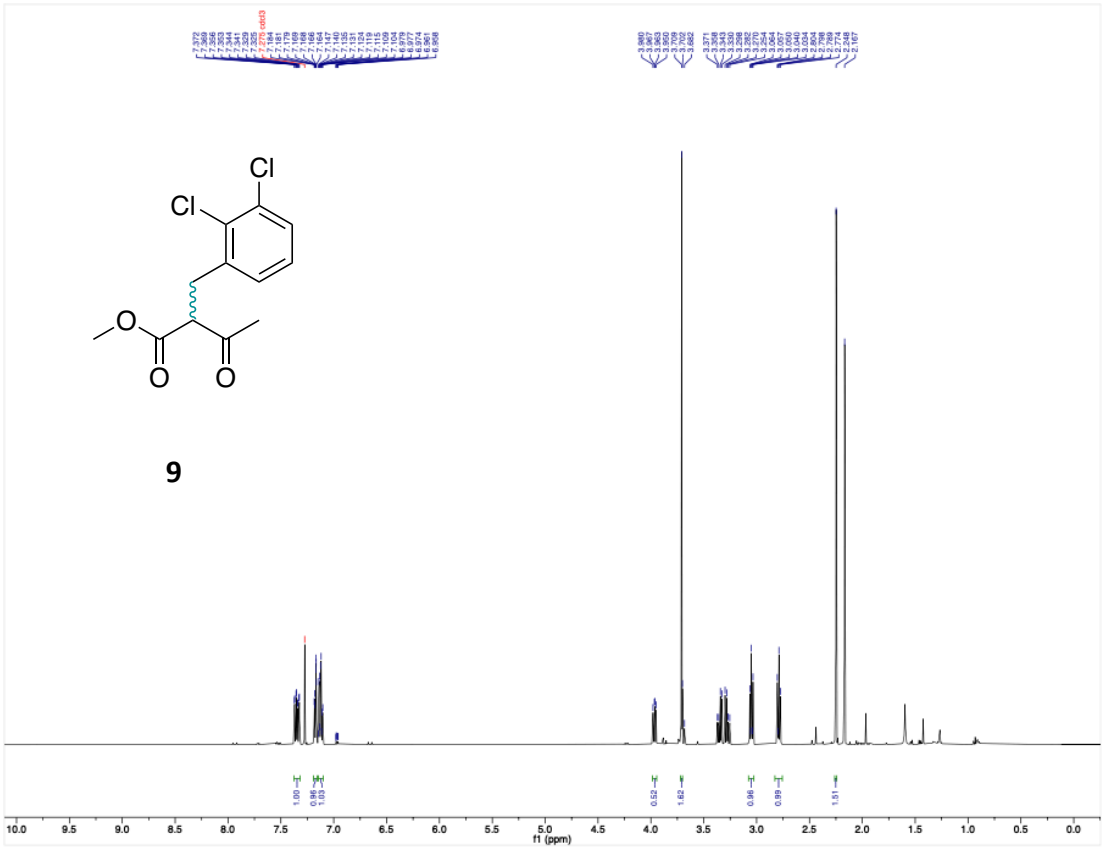
7

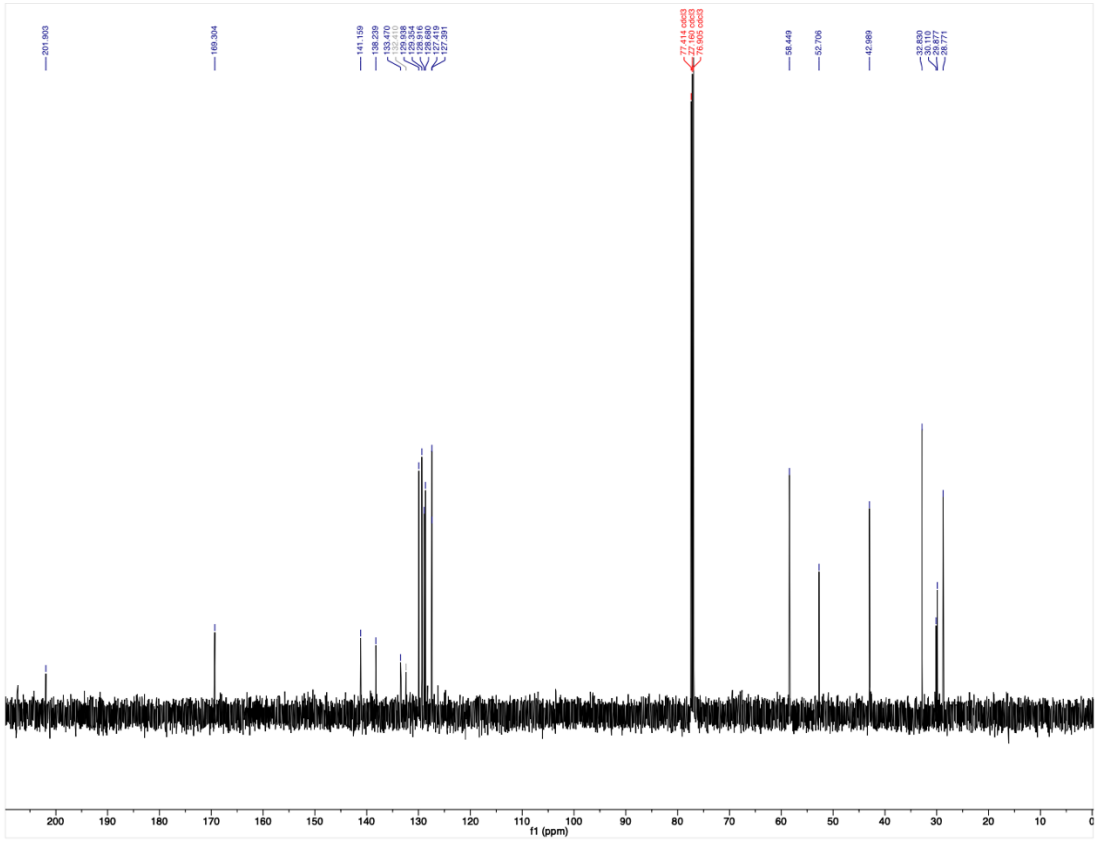


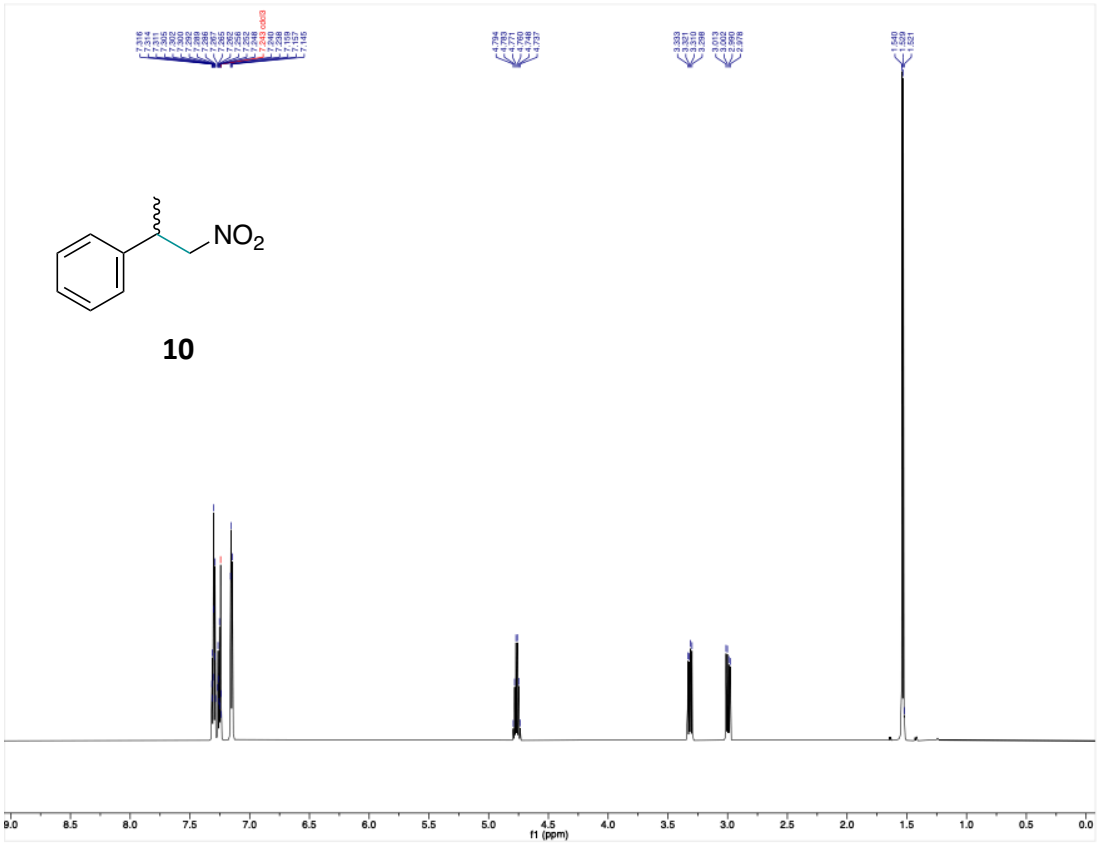


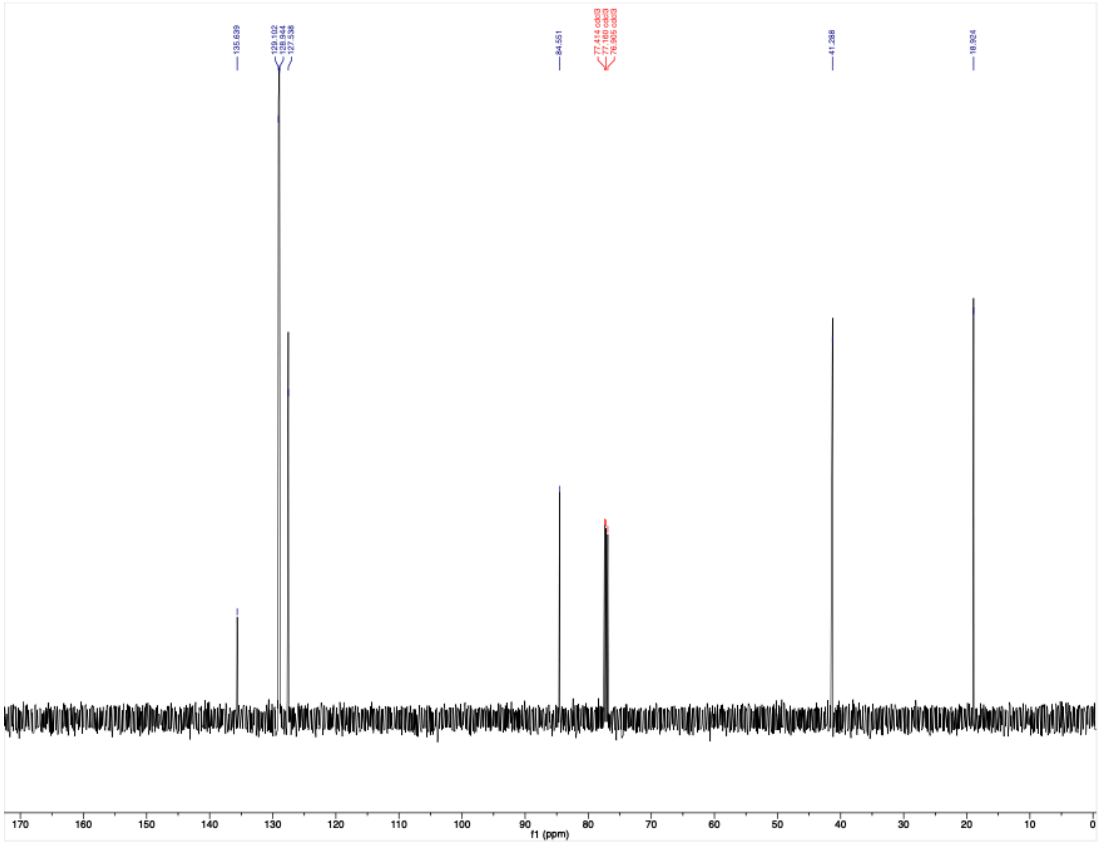
8

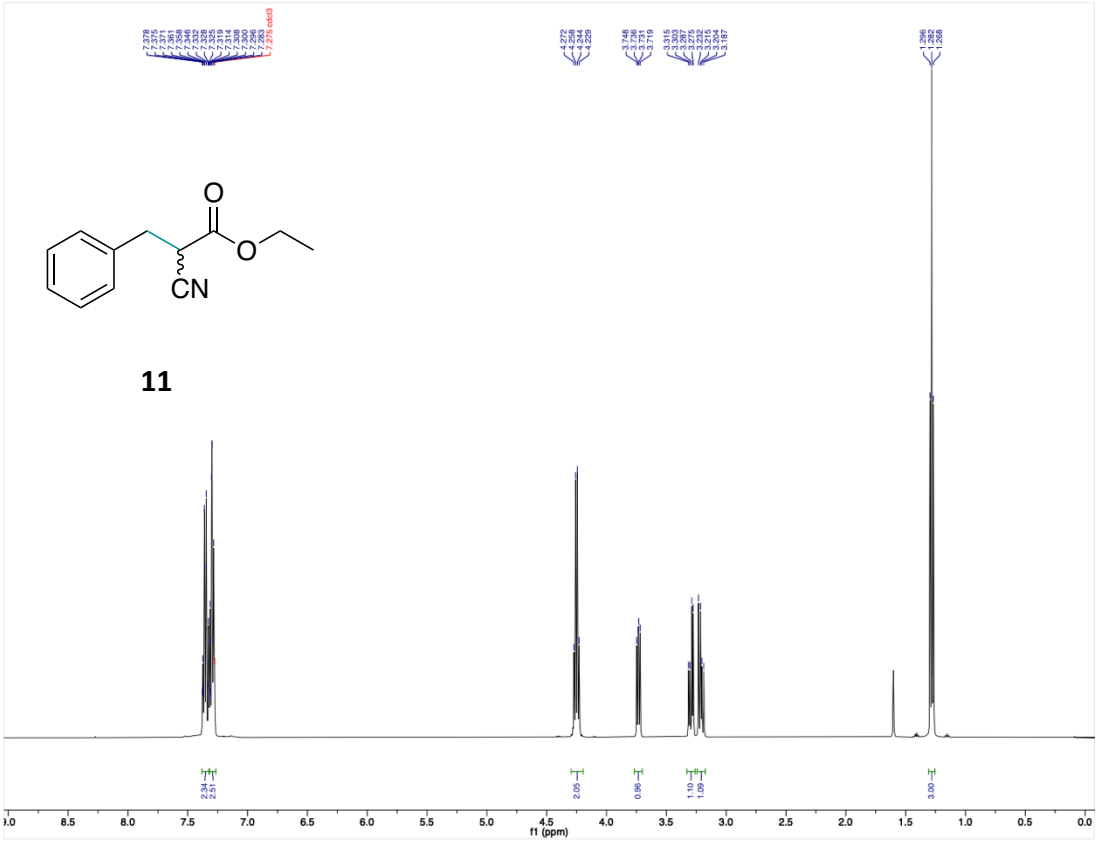



Cl
Cl
O
O
O
9
f1 (ppm)

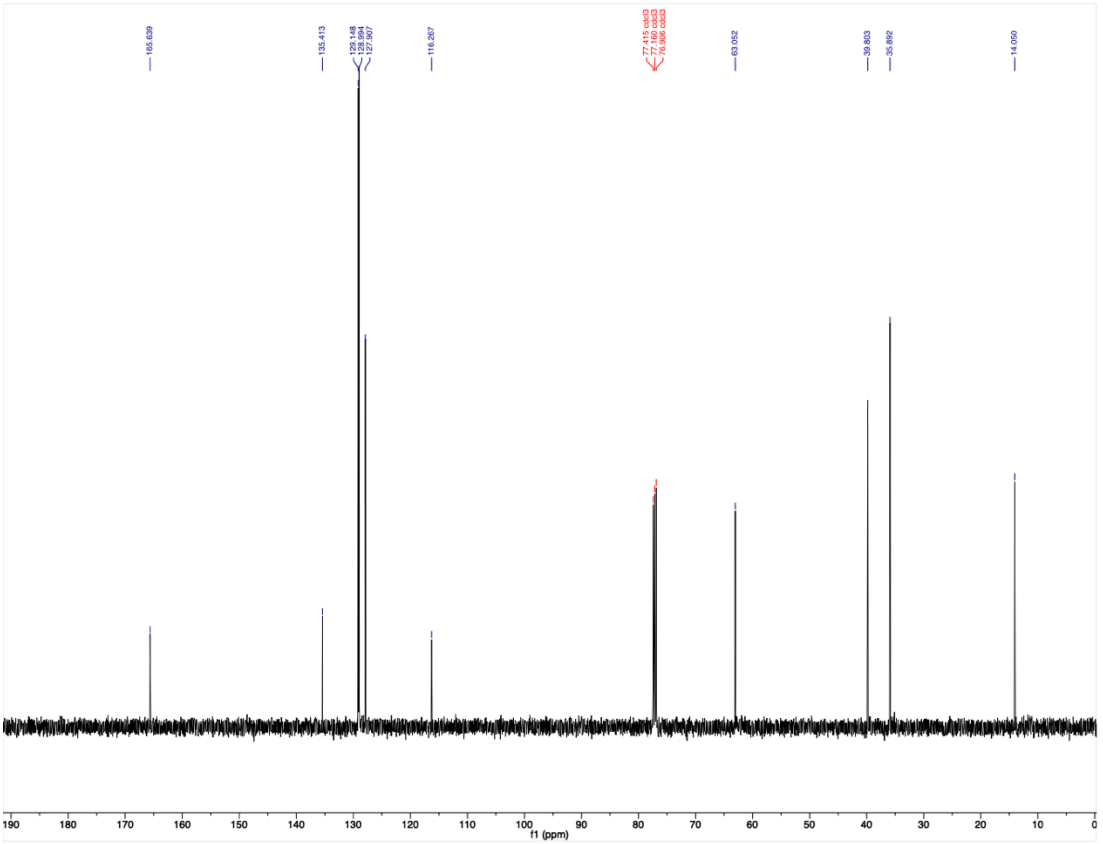

f1 (ppm)

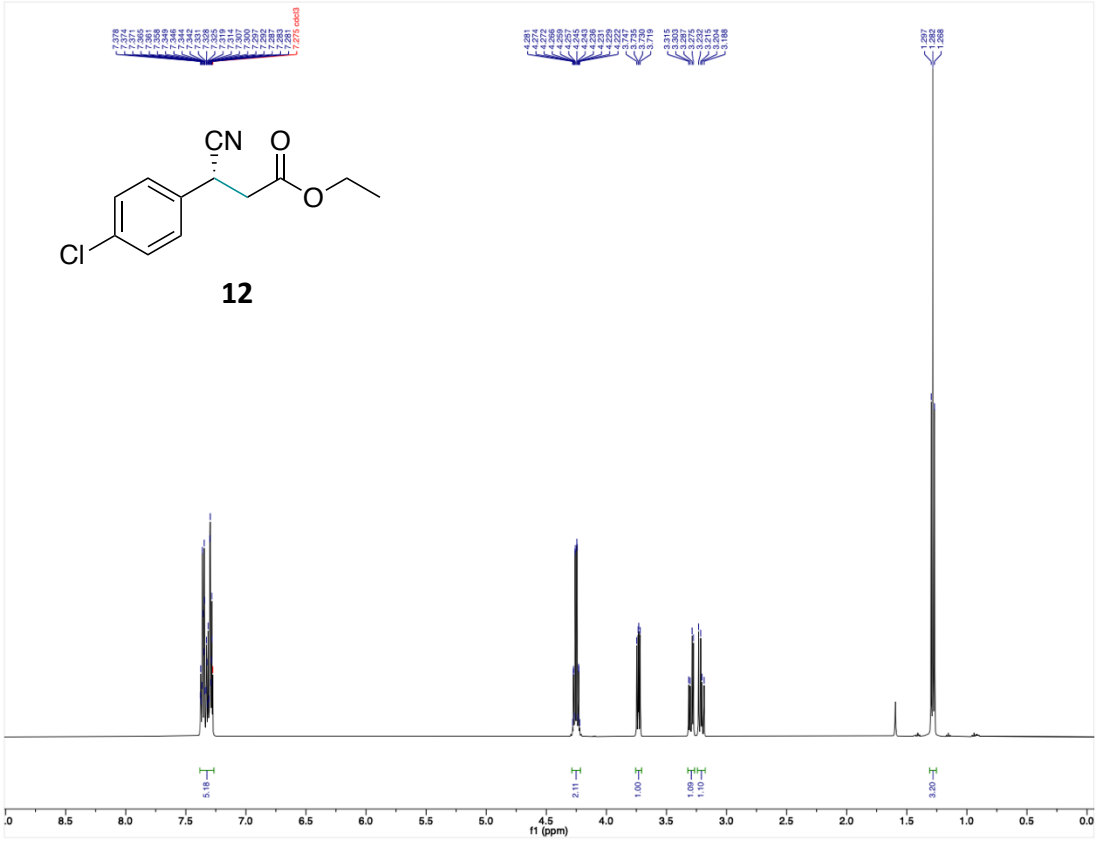

NO2
10
f1 (ppm)

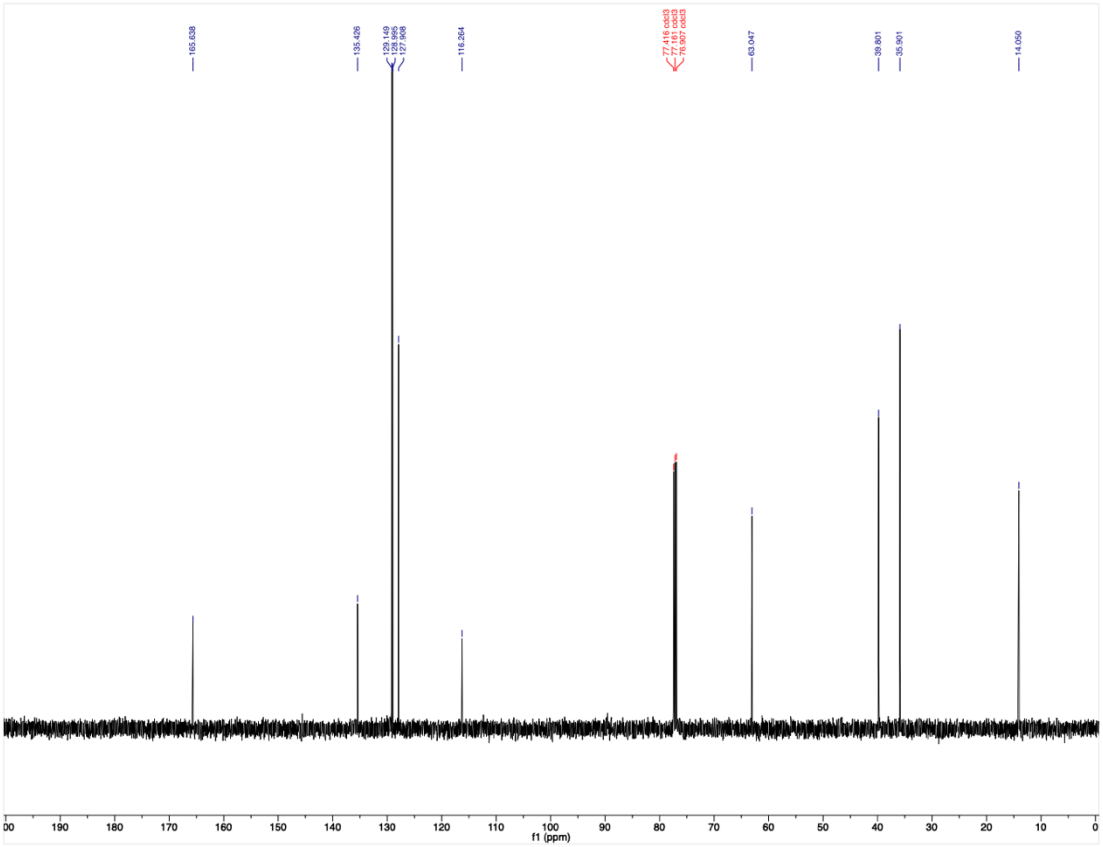

f1 (ppm)

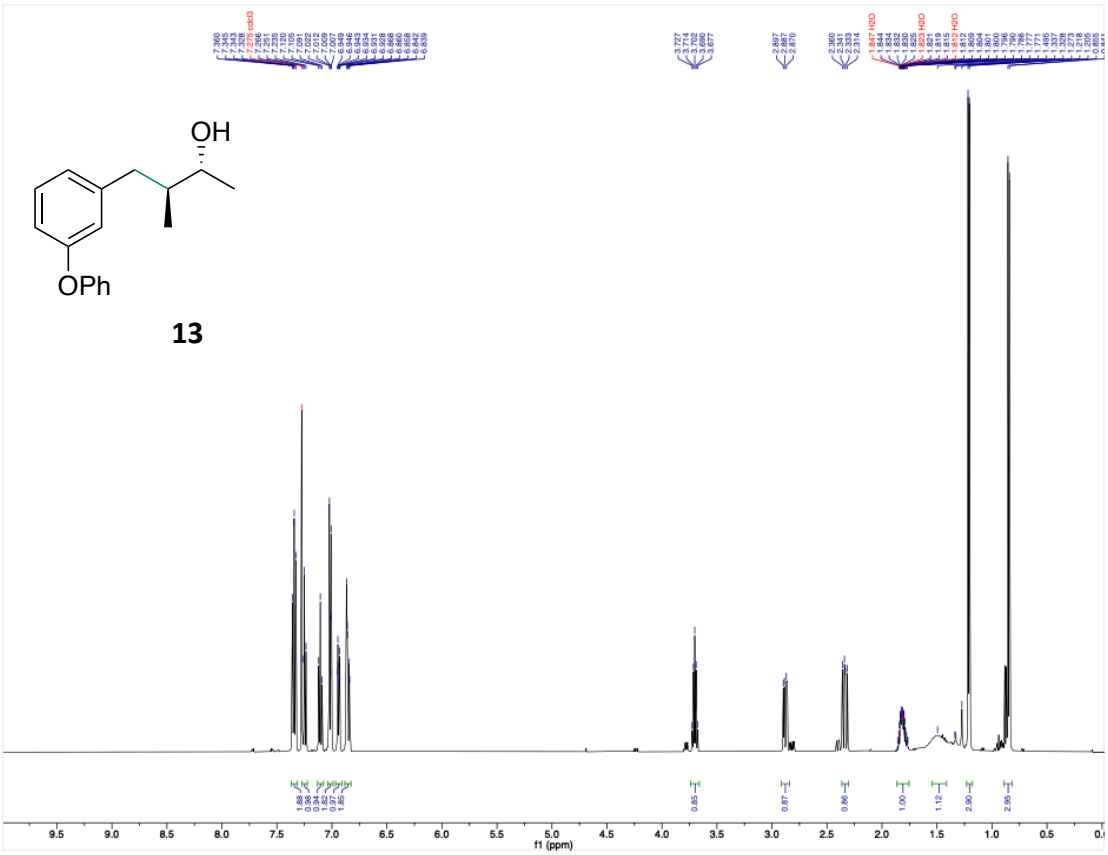


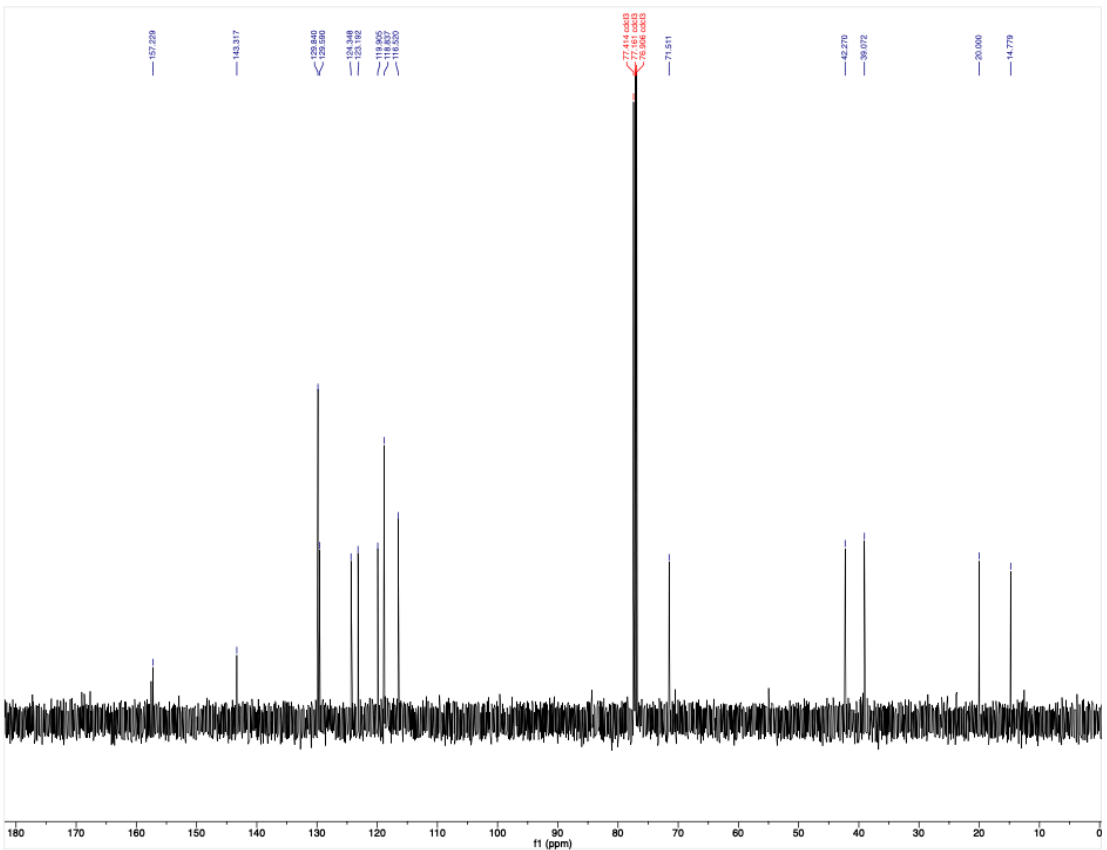
11

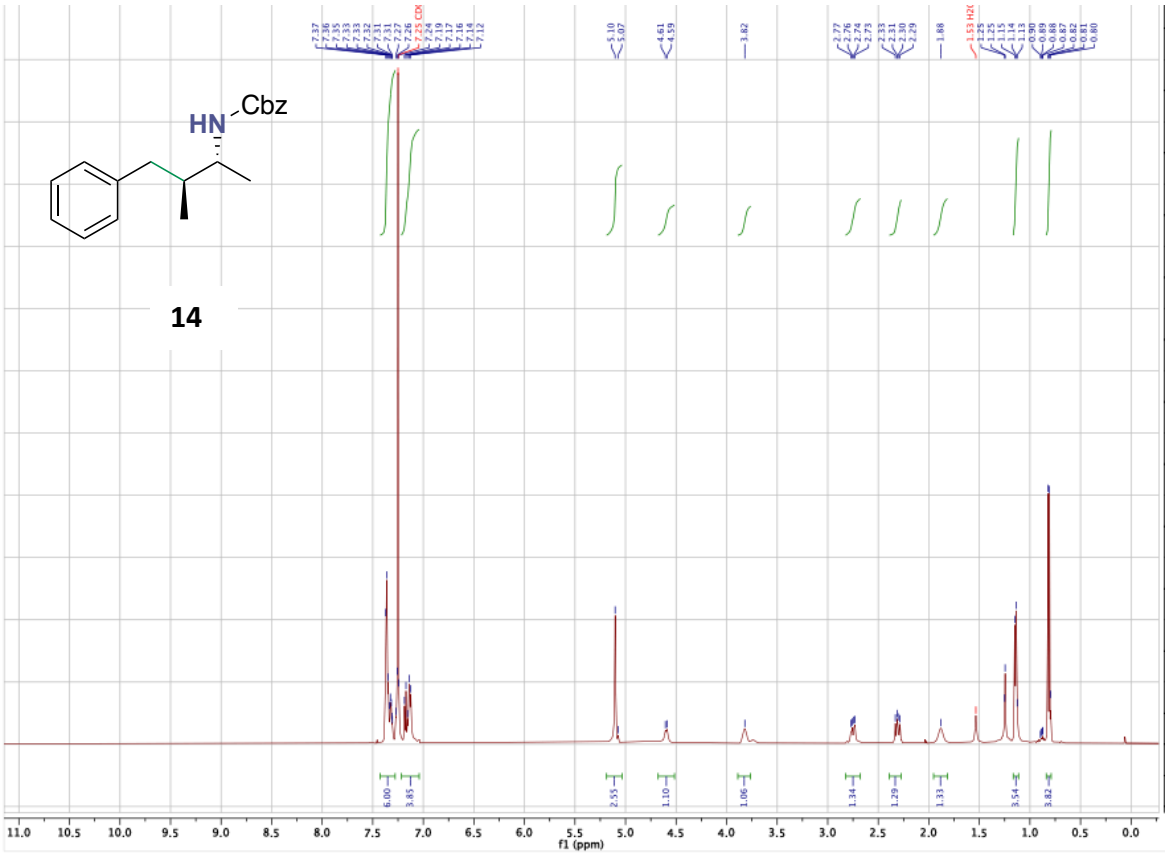


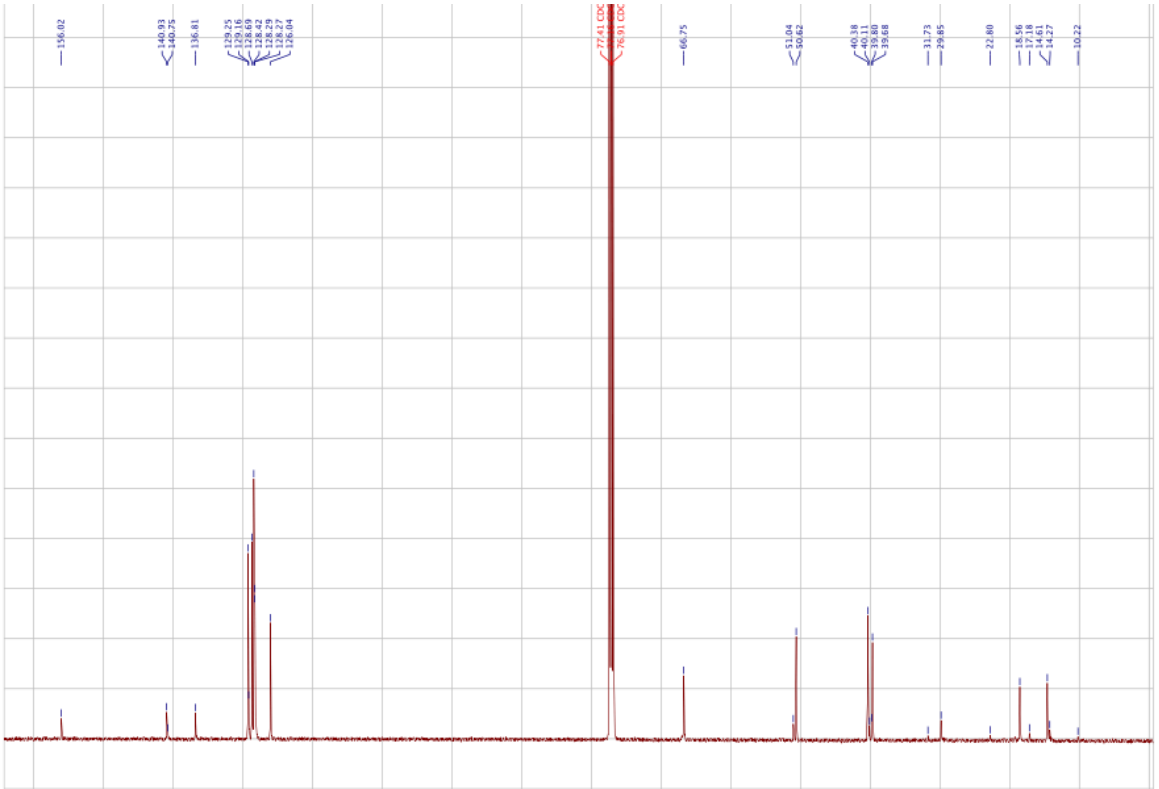

CN
O
O
Cl
12
f1 (ppm)

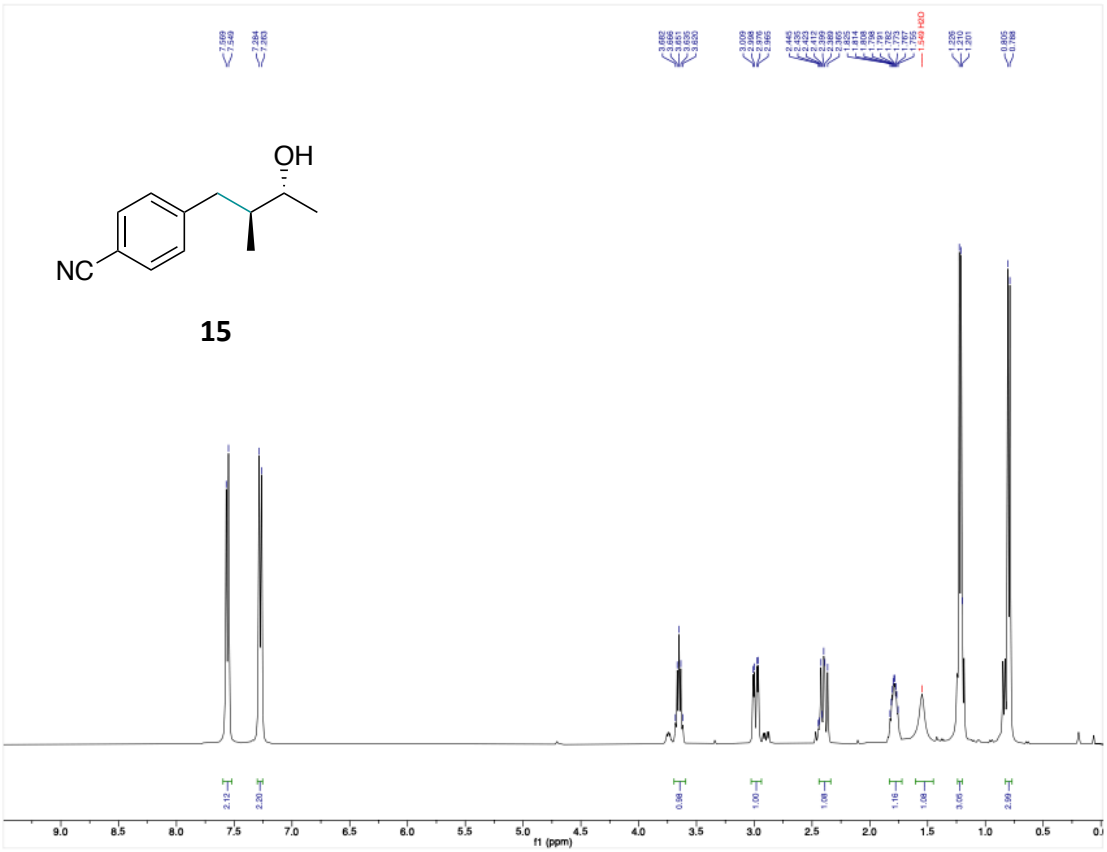

f1 (ppm)

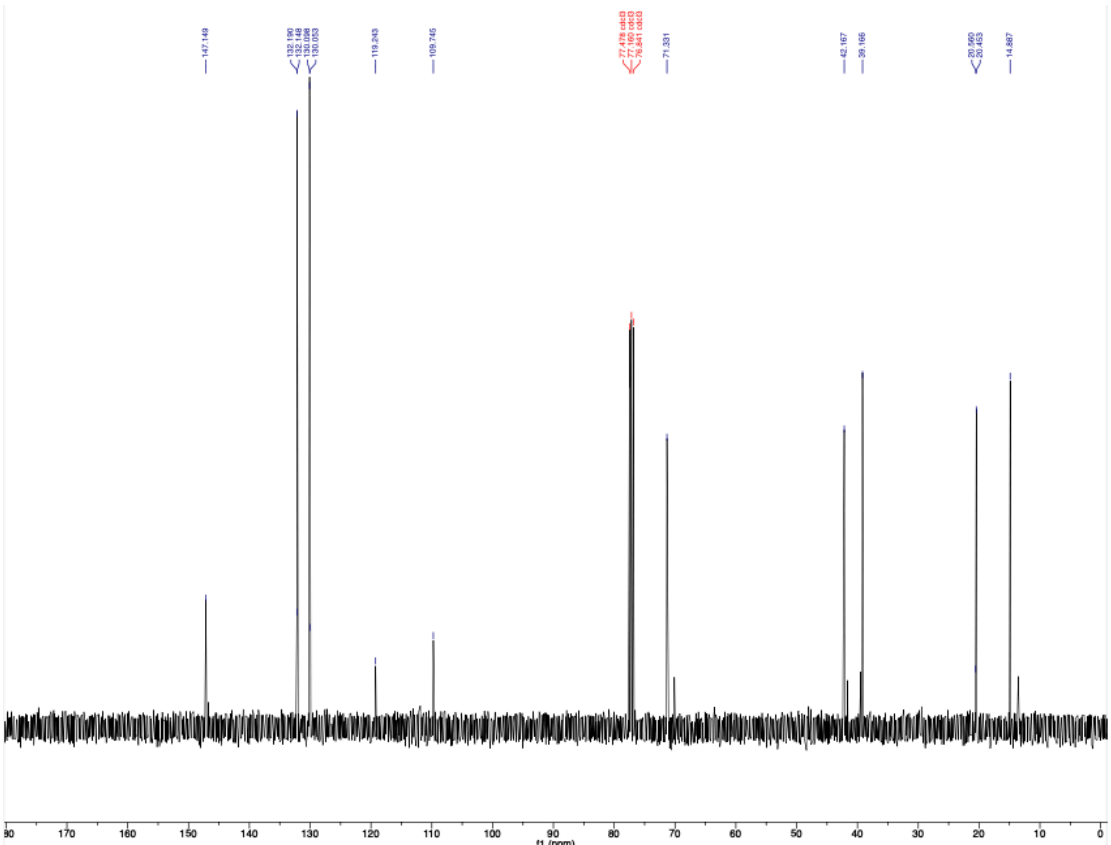

OH
OPh
13
f1 (ppm)

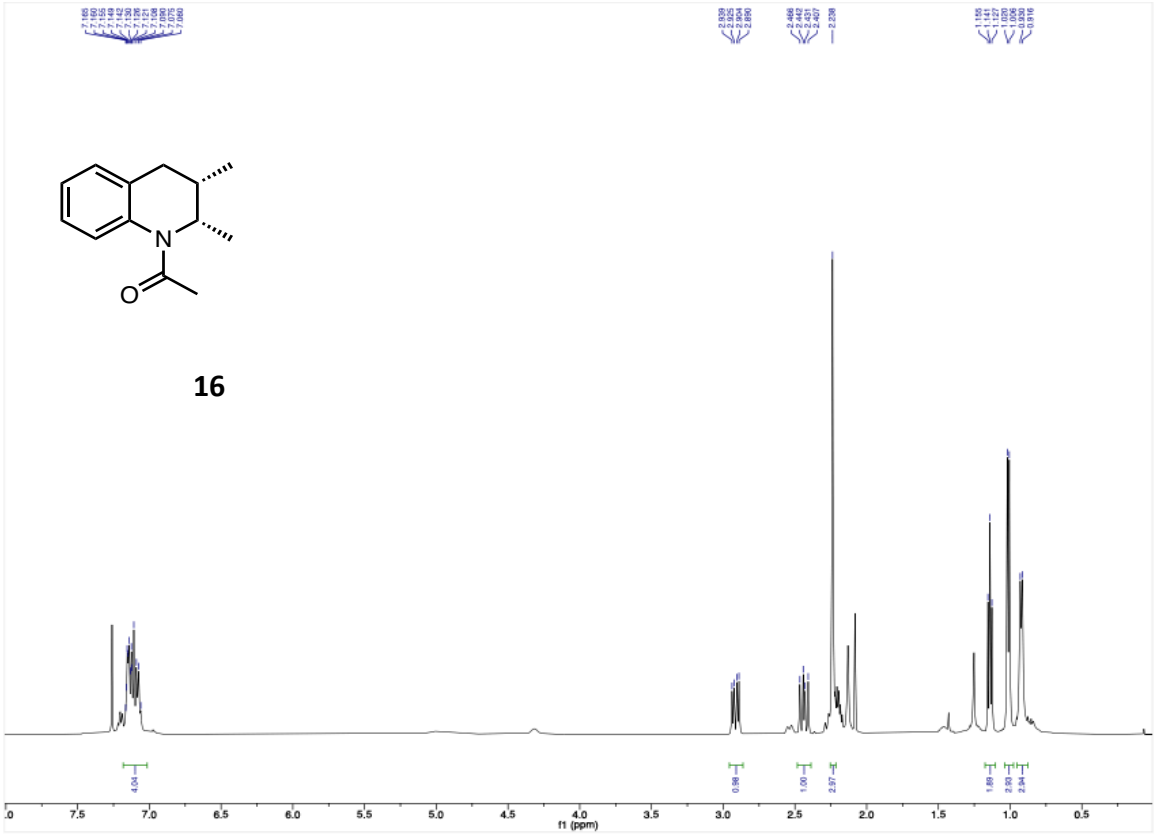

f1 (ppm)


HN
Cbz
14
f1 (ppm)

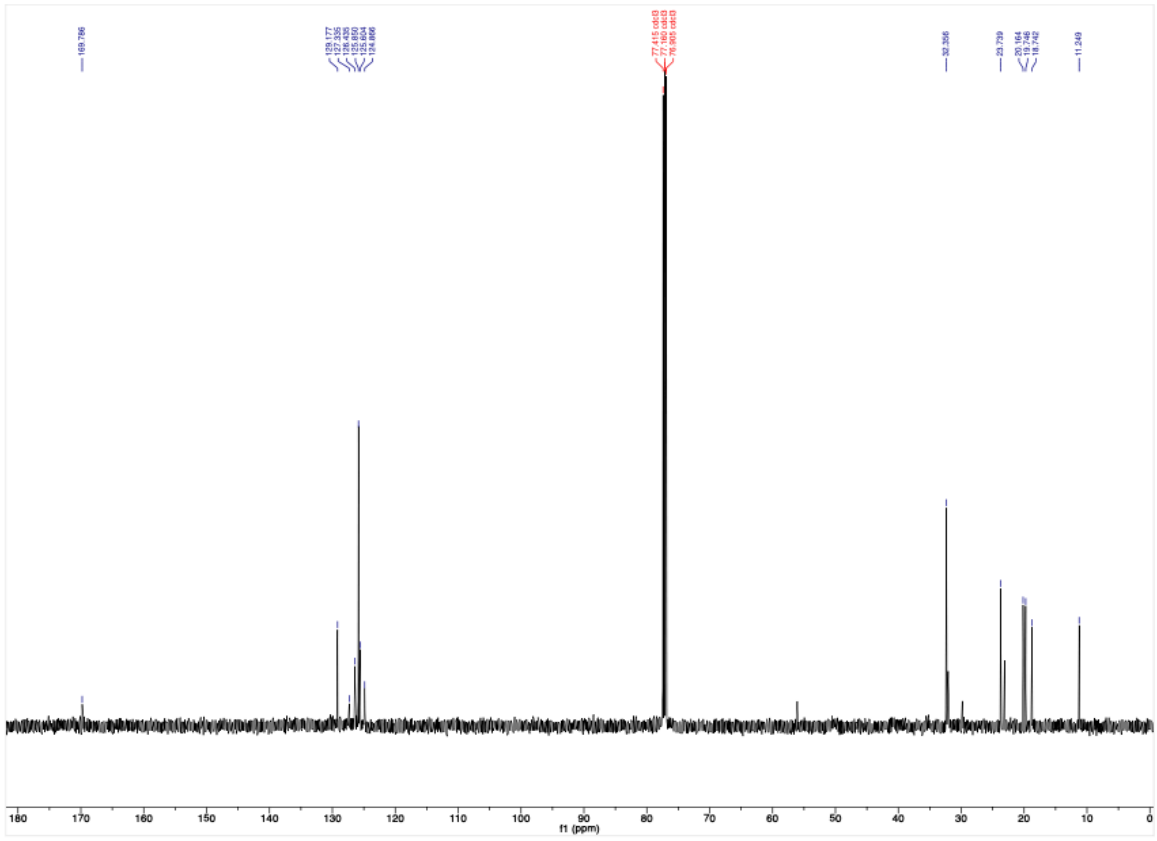



OH
NC
15
f1 (ppm)

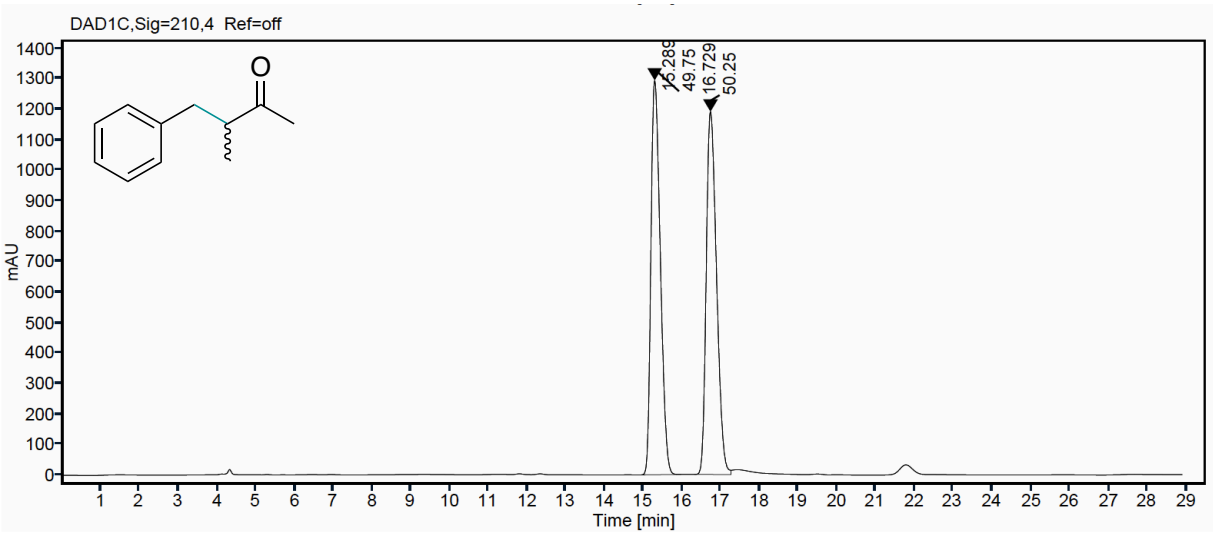

f1 (ppm)



16

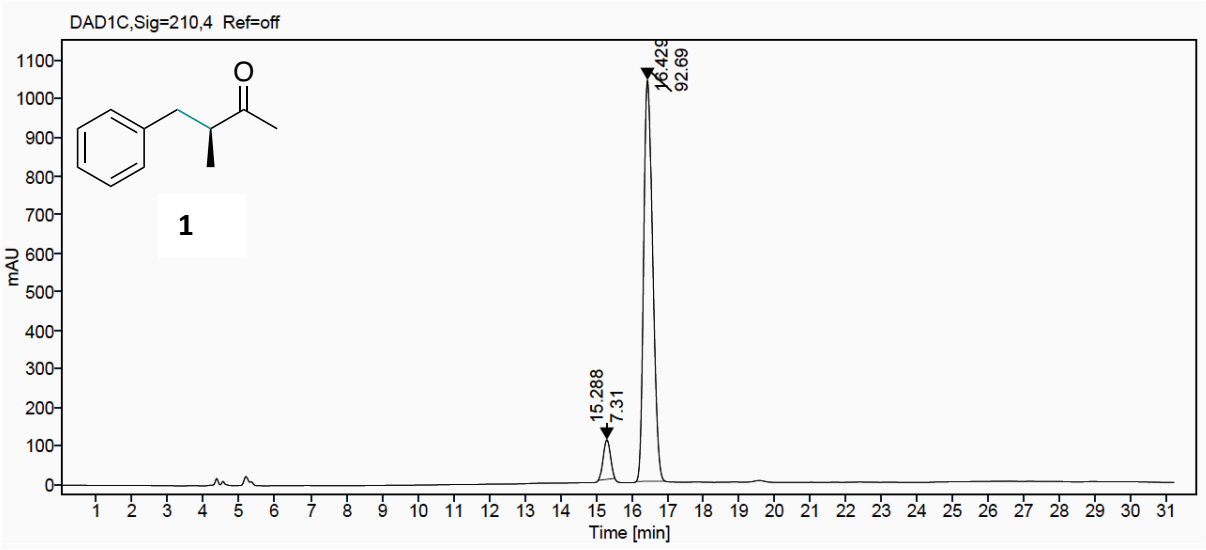


HPLC Data of Compounds



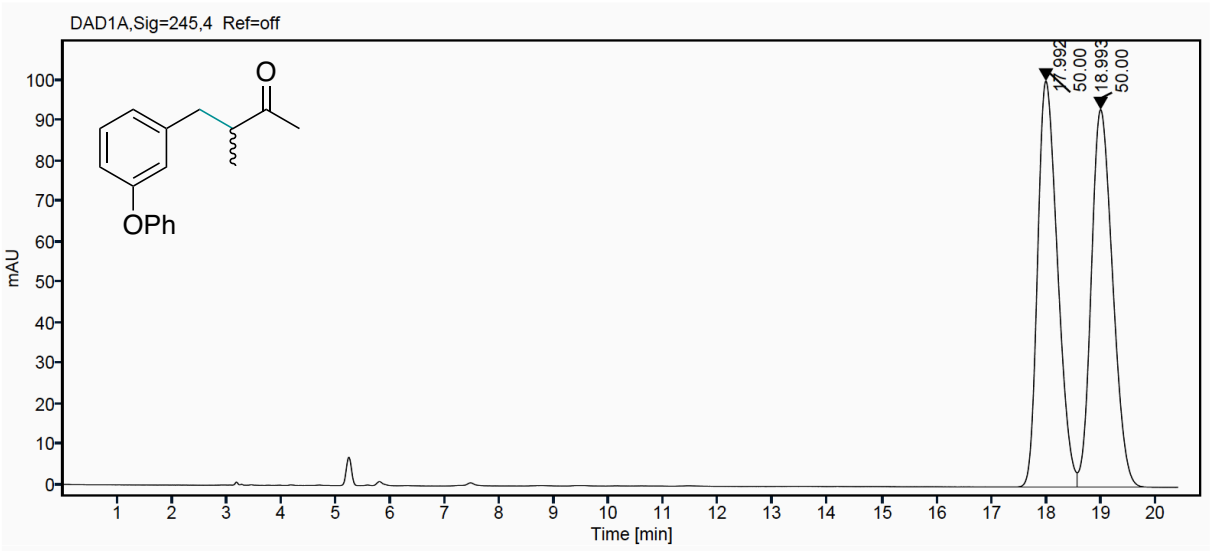
Signal: DAD1C,Sig=210,4 Ref=off

RT [min]	Area	Area%
15.289	21702.8687	49.7475
16.729	21923.1576	50.2525
Sum	43626.0263	



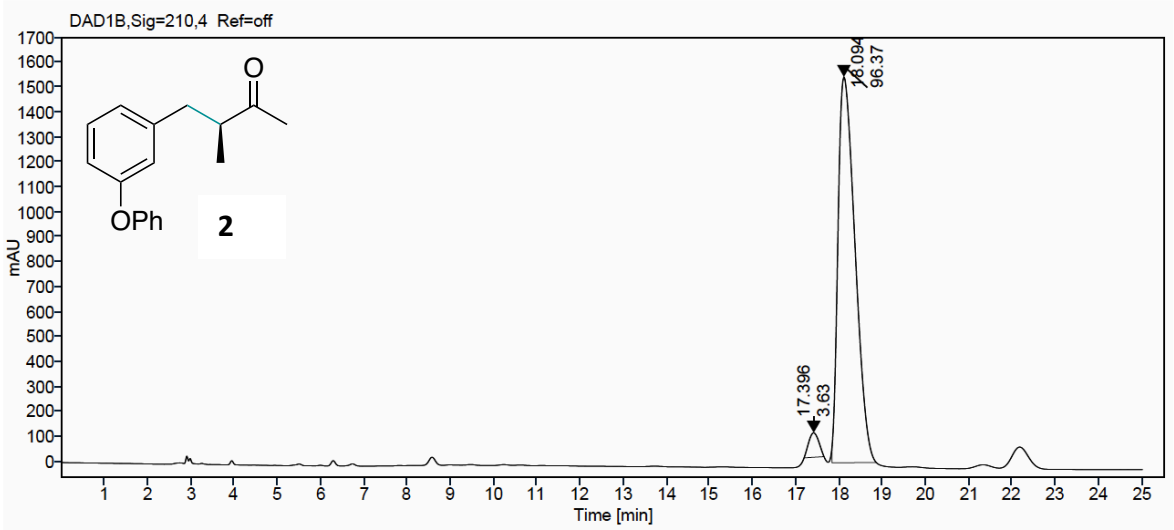
Signal: DAD1C,Sig=210,4 Ref=off

RT [min]	Area	Area%
15.288	1438.2339	7.3052
16.429	18249.6836	92.6948
Sum	19687.9176	



Signal: DAD1A,Sig=245,4 Ref=off

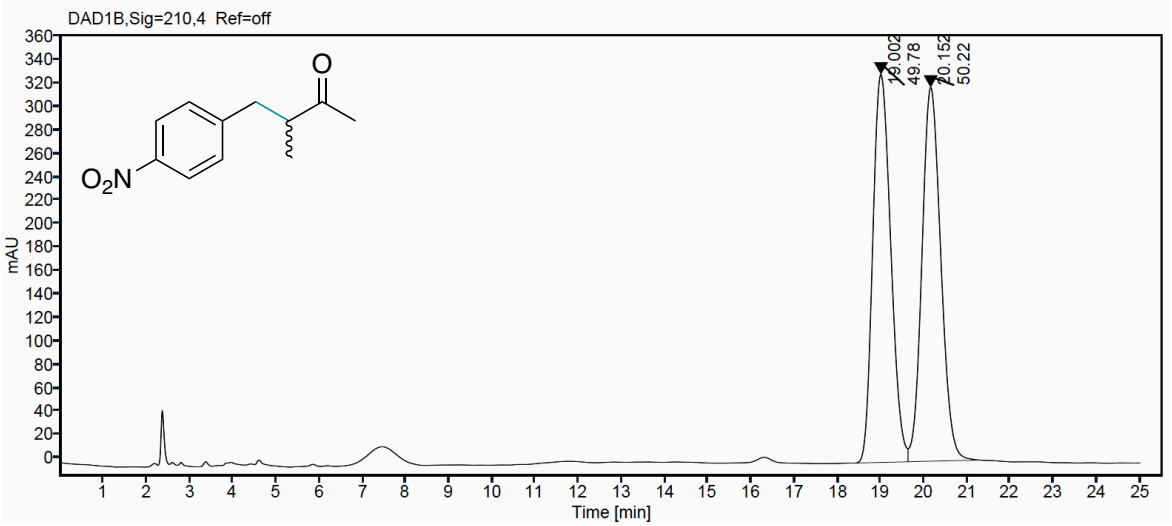
RT [min]	Area	Area%
17.992	2532.8344	50.0046
18.993	2532.3725	49.9954
Sum	5065.2069	



Signal: DAD1B,Sig=210,4 Ref=off

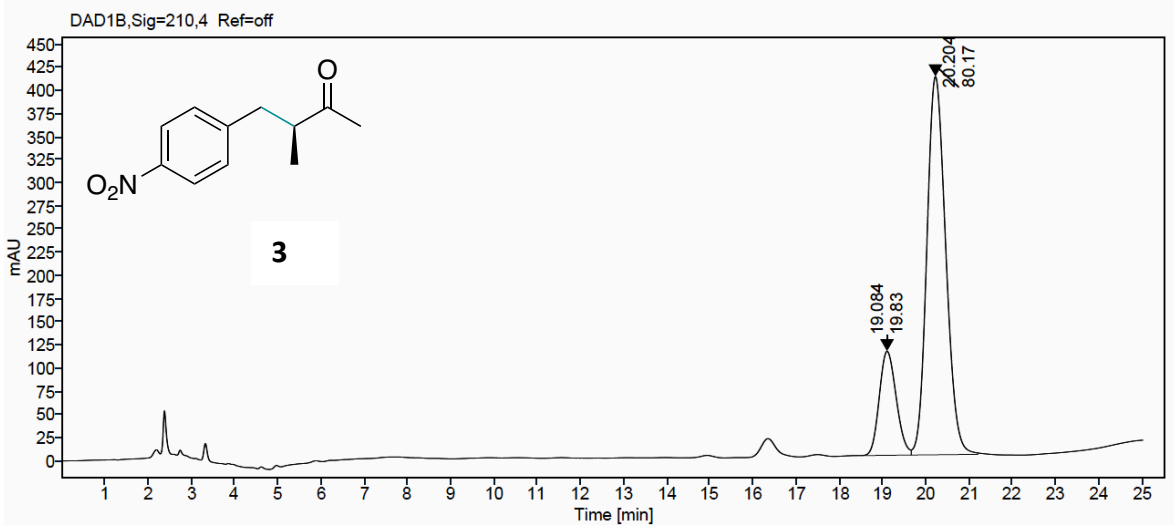
RT [min]	Area	Area%
17.396	1587.6900	3.6271
18.094	42185.2490	96.3729
Sum	43772.9390	

The retention times of the racemate and the product almost align. It is slightly shifted by 0.8 mins.



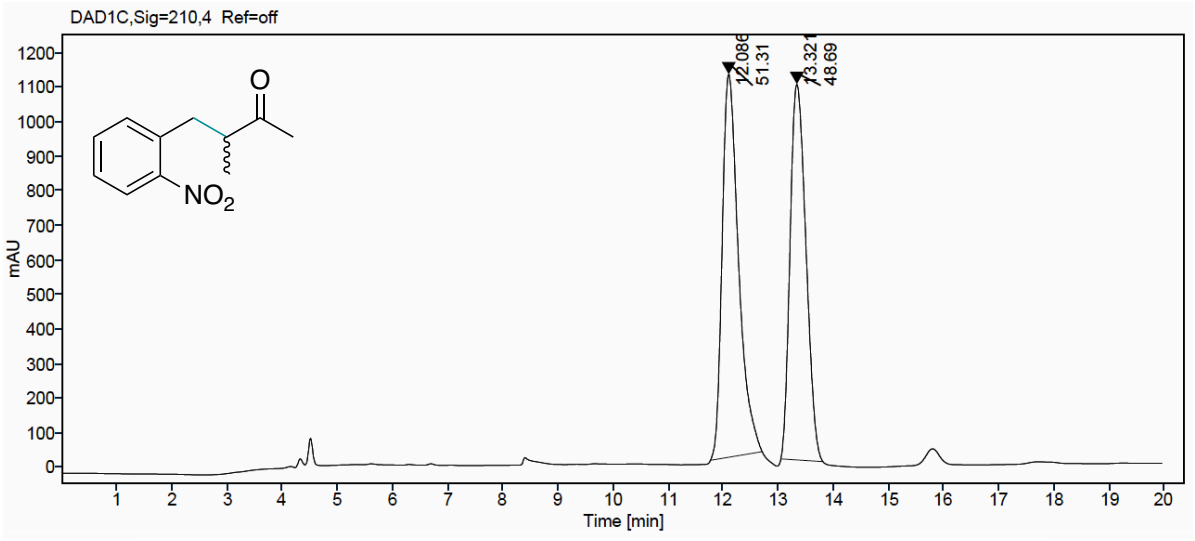
Signal: DAD1B,Sig=210,4 Ref=off

RT [min]	Area	Area%
19.002	9574.4207	49.7827
20.152	9658.0165	50.2173
Sum	19232.4372	



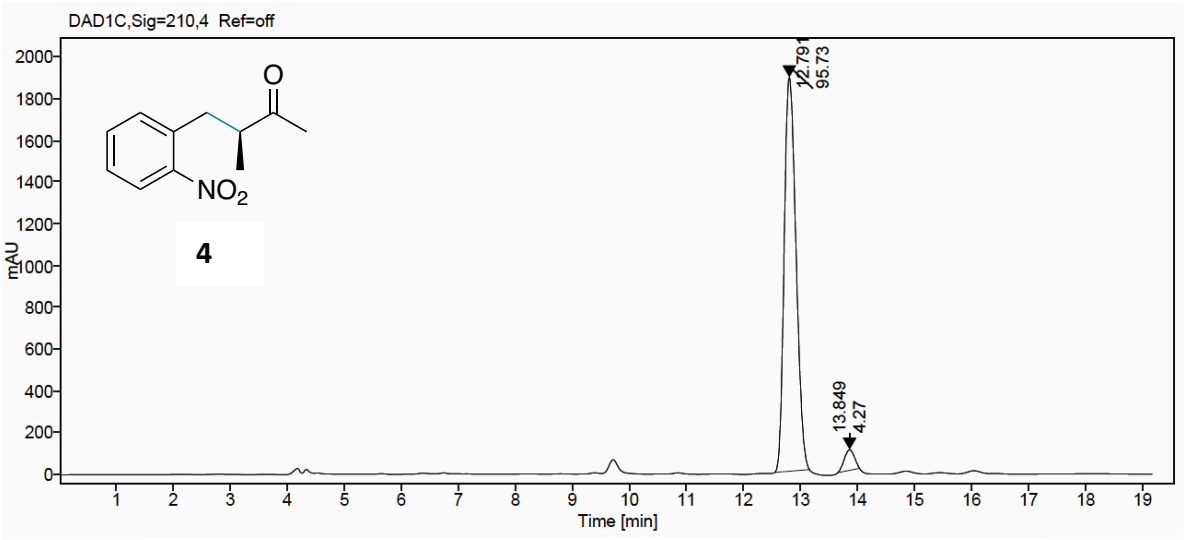
Signal: DAD1B,Sig=210,4 Ref=off

RT [min]	Area	Area%
19.084	3065.7096	19.8309
20.204	12393.5593	80.1691
Sum	15459.2689	



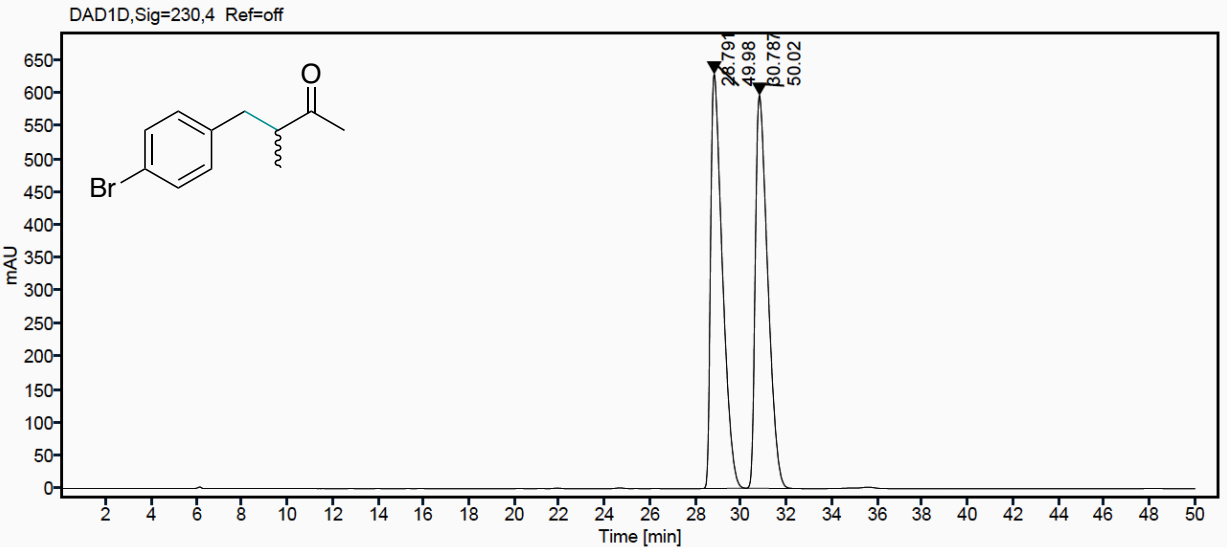
Signal: DAD1C,Sig=210,4 Ref=off

RT [min]	Area	Area%
12.086	22872.5599	51.3058
13.321	21708.2743	48.6942
Sum	44580.8343	



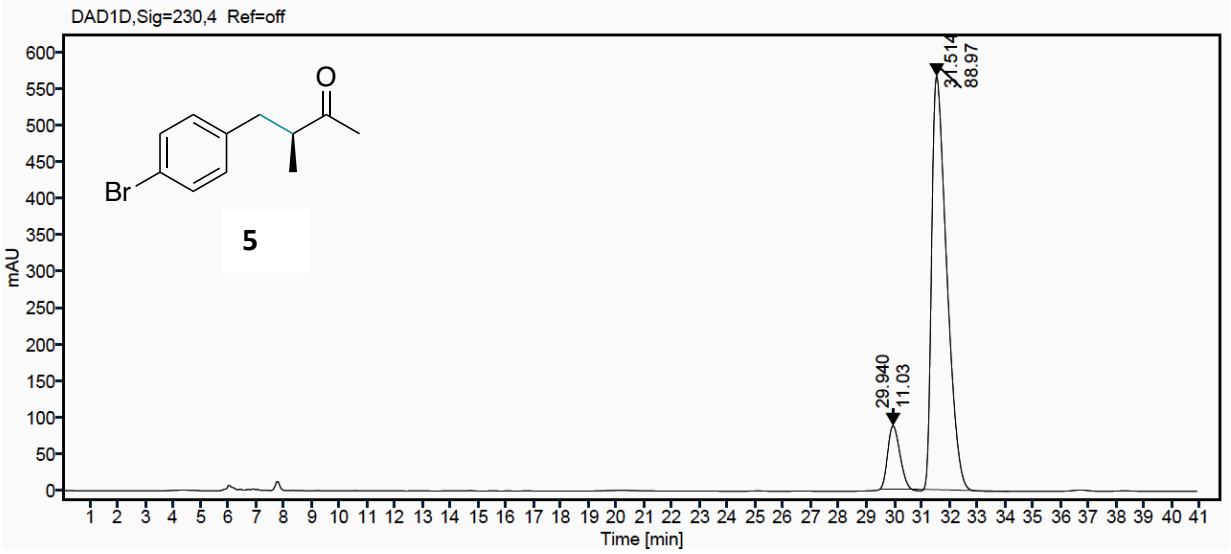
Signal: DAD1C,Sig=210,4 Ref=off

RT [min]	Area	Area%
12.791	27124.3557	95.7291
13.849	1210.1524	4.2709
Sum	28334.5082	



Signal: DAD1D,Sig=230,4 Ref=off

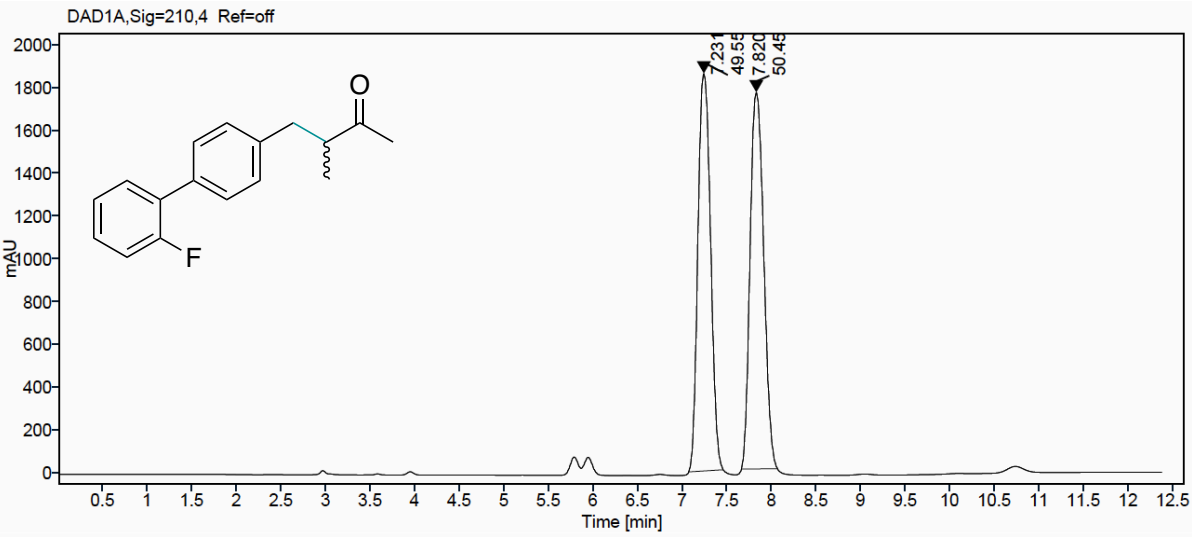
RT [min]	Area	Area%
28.791	22751.7105	49.9755
30.787	22774.0037	50.0245
Sum	45525.7142	



Signal: DAD1D,Sig=230,4 Ref=off

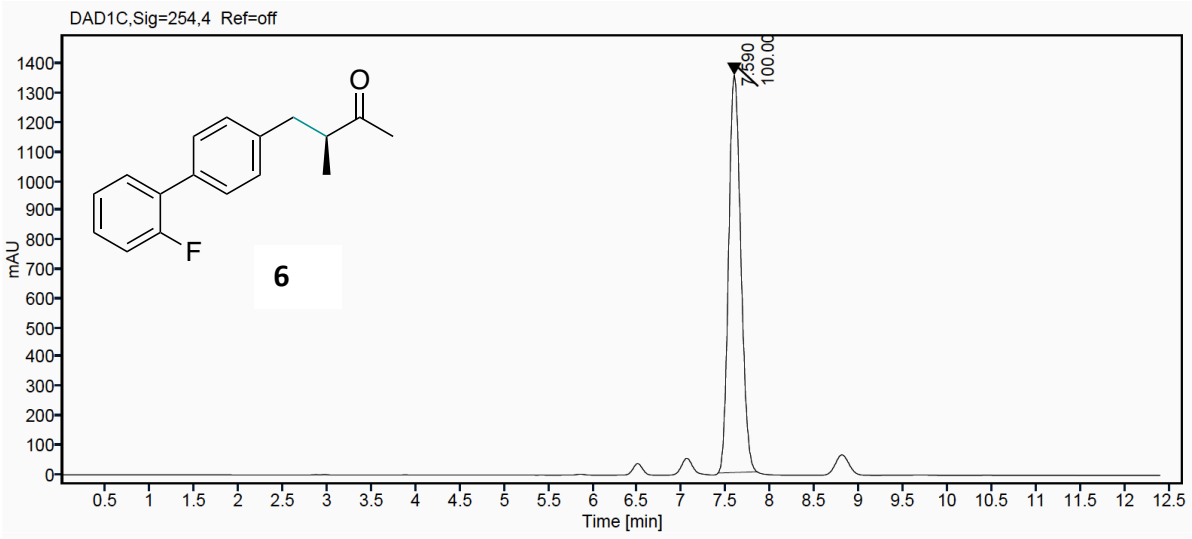
RT [min]	Area	Area%
29.940	2639.6488	11.0313
31.514	21289.1329	88.9687
Sum	23928.7818	

The retention times of the racemate and the product almost align. It is slightly shifted by 1 min.



Signal: DAD1A,Sig=210,4 Ref=off

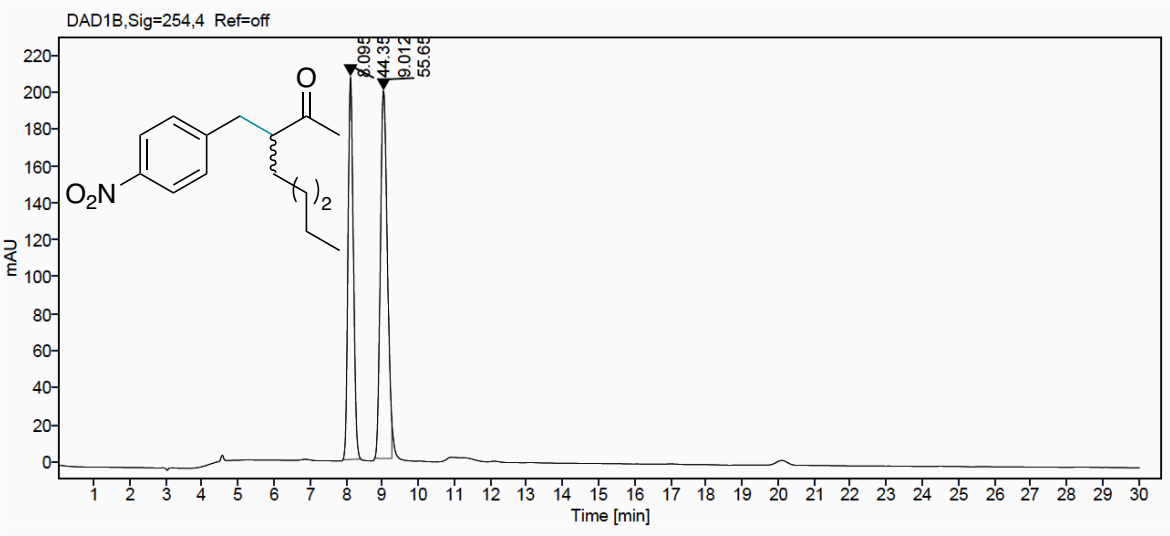
RT [min]	Area	Area%
7.231	18289.8210	49.5458
7.820	18625.1391	50.4542
Sum	36914.9601	



Signal: DAD1C,Sig=254,4 Ref=off

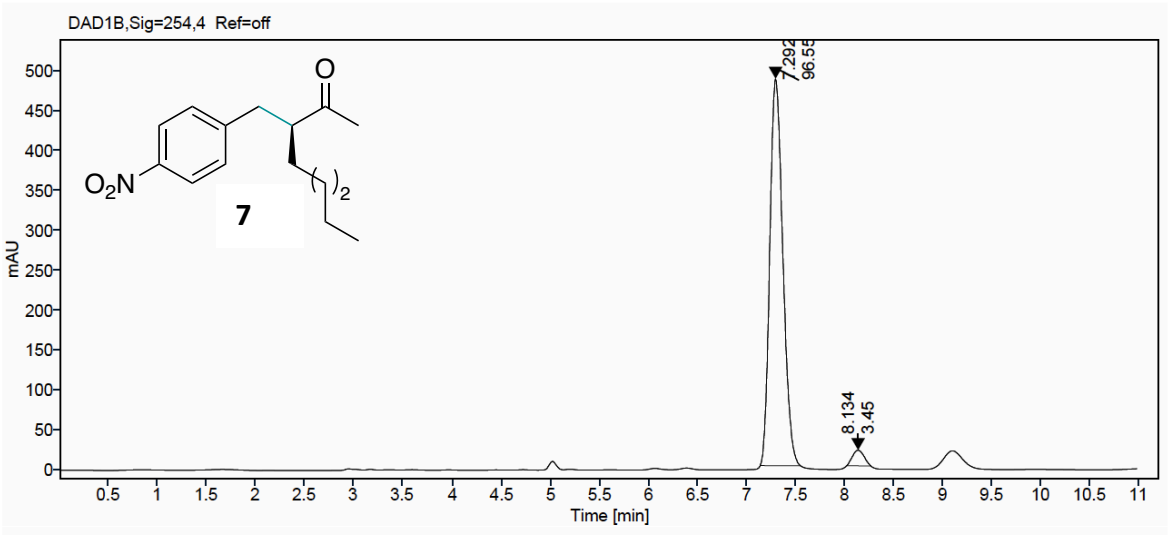
RT [min]	Area	Area%
7.590	12974.6033	100.0000
Sum	12974.6033	

The small peak around 7 min is not an enantiomer. It is a small impurity that has an area of <1%.



Signal: DAD1B,Sig=254,4 Ref=off

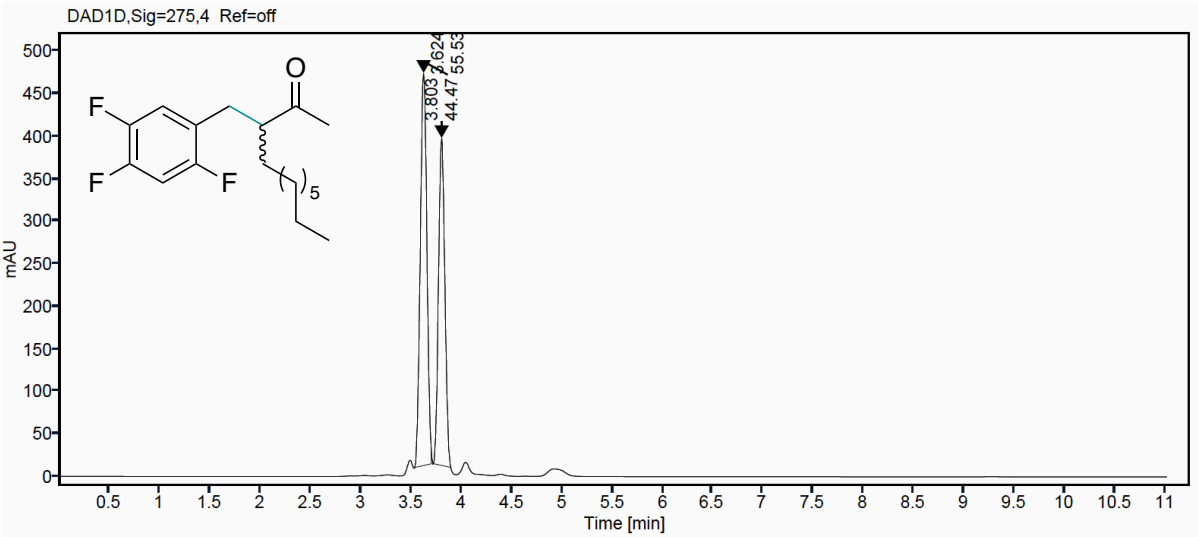
RT [min]	Area	Area%
8.095	2129.4370	44.3478
9.012	2672.2338	55.6522
Sum	4801.6709	



Signal: DAD1B,Sig=254,4 Ref=off

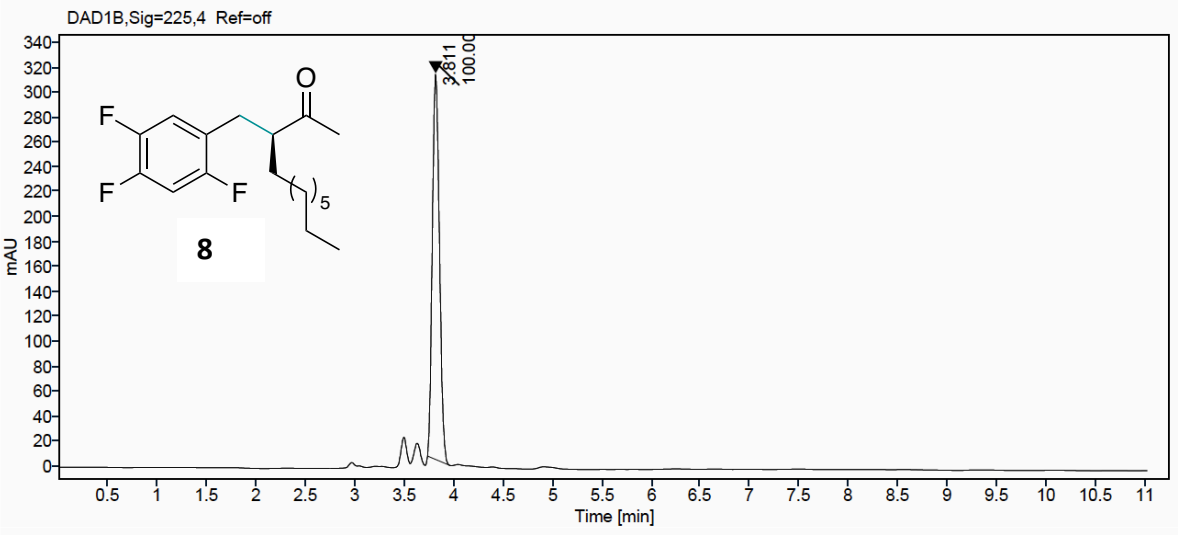
RT [min]	Area	Area%
7.292	4464.8899	96.5469
8.134	159.6927	3.4531
Sum	4624.5827	

The retention times of the racemate and the product almost align. It is slightly shifted by <0.8 mins.



Signal: DAD1D,Sig=275,4 Ref=off

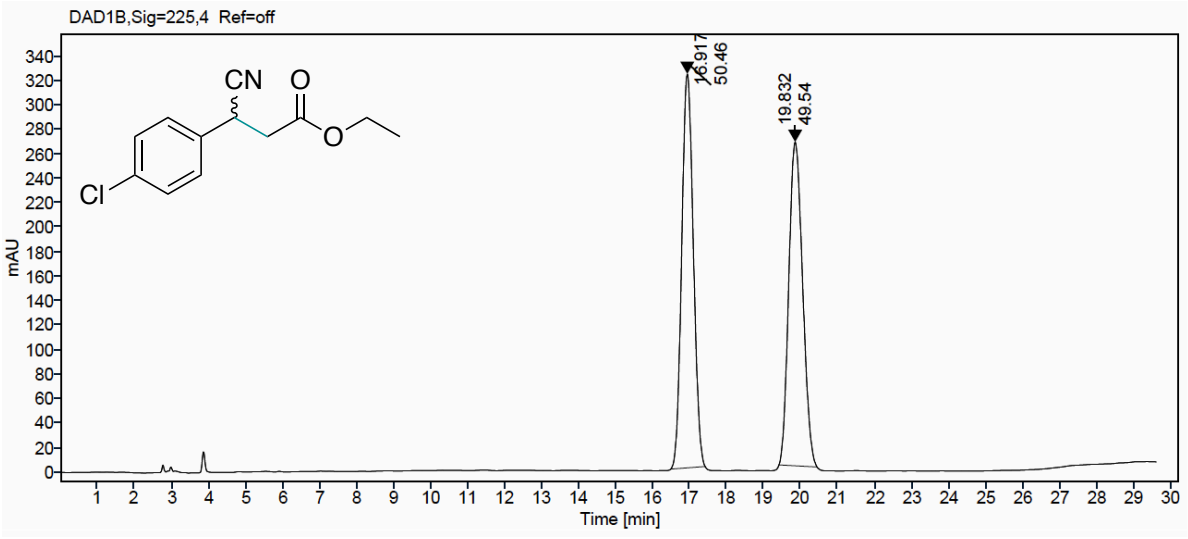
RT [min]	Area	Area%
3.624	2101.3499	55.5288
3.803	1682.9017	44.4712
Sum	3784.2516	



Signal: DAD1B,Sig=225,4 Ref=off

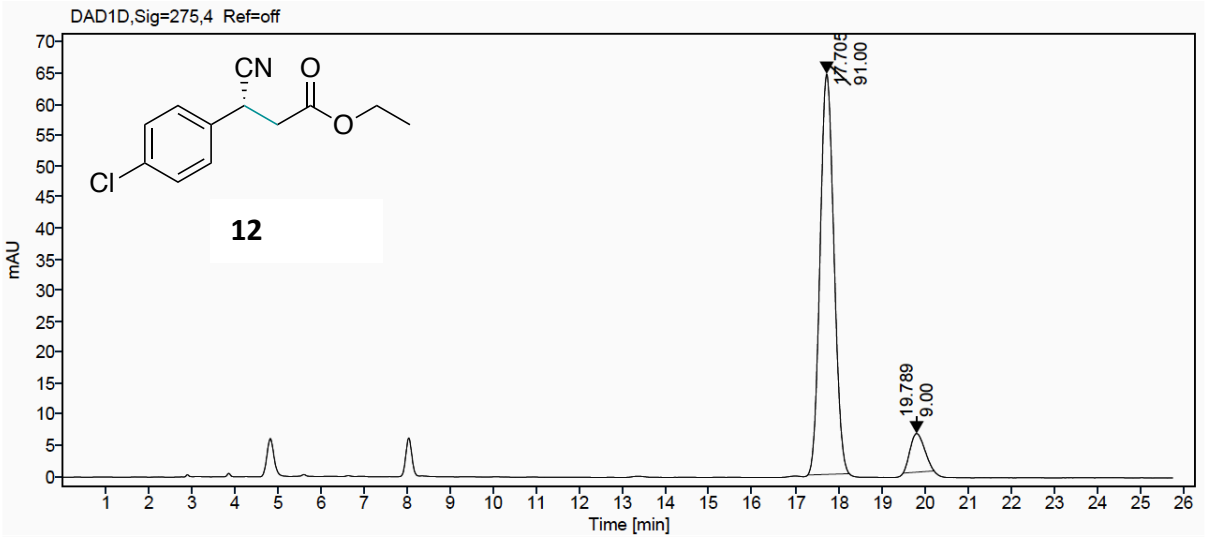
RT [min]	Area	Area%
3.811	1550.5153	100.0000
Sum	1550.5153	

The small peak around 3.5 min is not an enantiomer. It is small impurity that has an area of <1%.



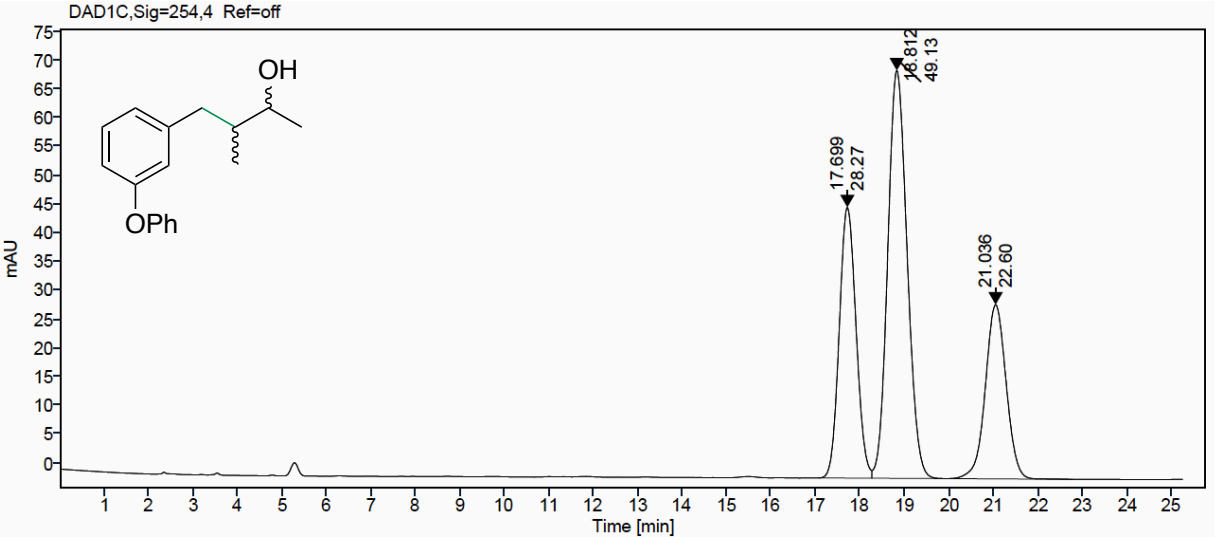
Signal: DAD1B,Sig=225,4 Ref=off

RT [min]	Area	Area%
16.917	6995.6657	50.4560
19.832	6869.2293	49.5440
Sum	13864.8950	



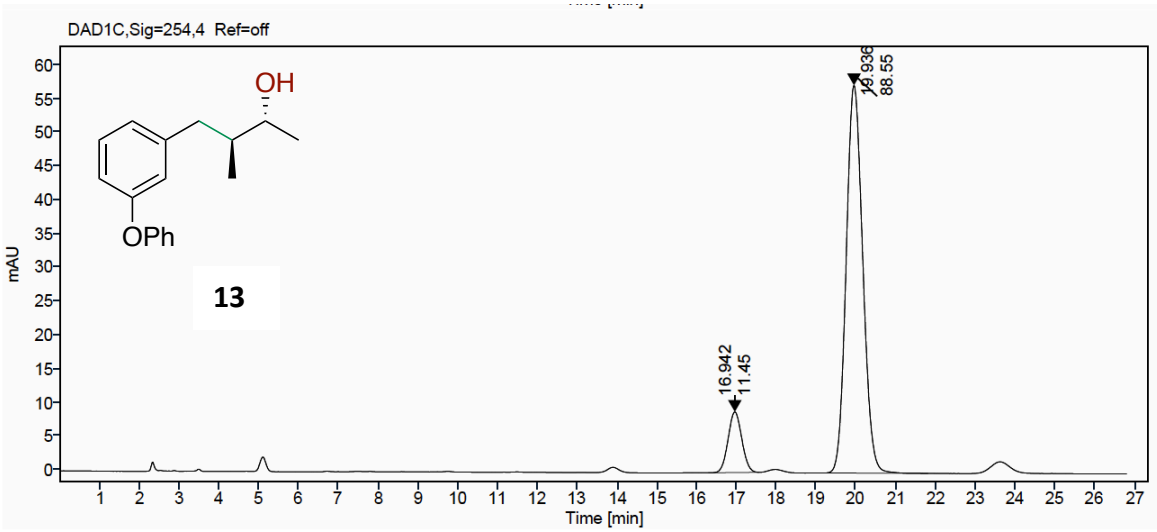
Signal: DAD1D,Sig=275,4 Ref=off

RT [min]	Area	Area%
17.705	1441.0306	91.0005
19.789	142.5111	8.9995
Sum	1583.5417	



Signal: DAD1C,Sig=254,4 Ref=off

RT [min]	Area	Area%
17.699	1235.8353	28.2703
18.812	2147.5742	49.1267
21.036	988.0926	22.6030
Sum	4371.5021	

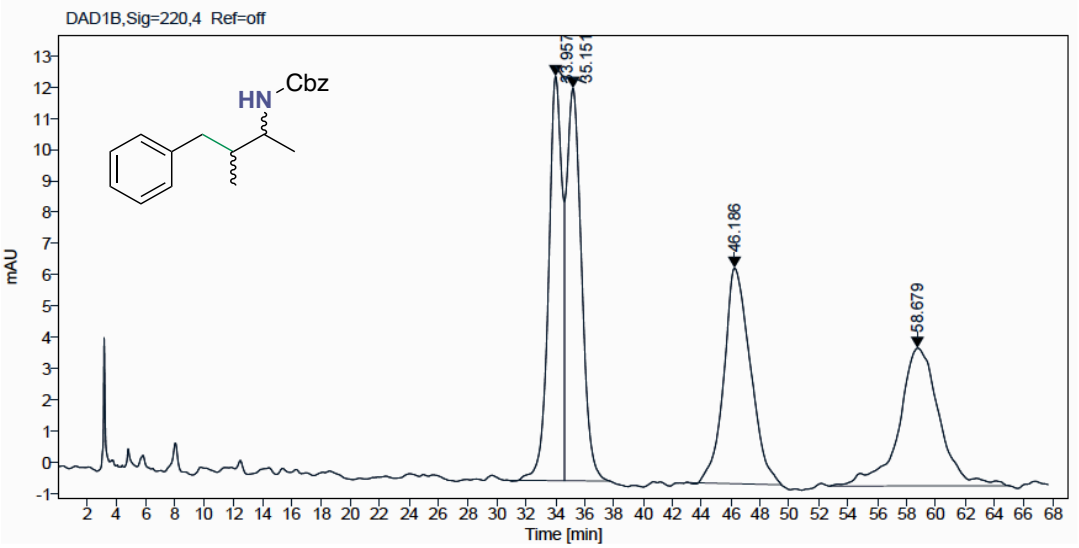


Signal: DAD1C,Sig=254,4 Ref=off

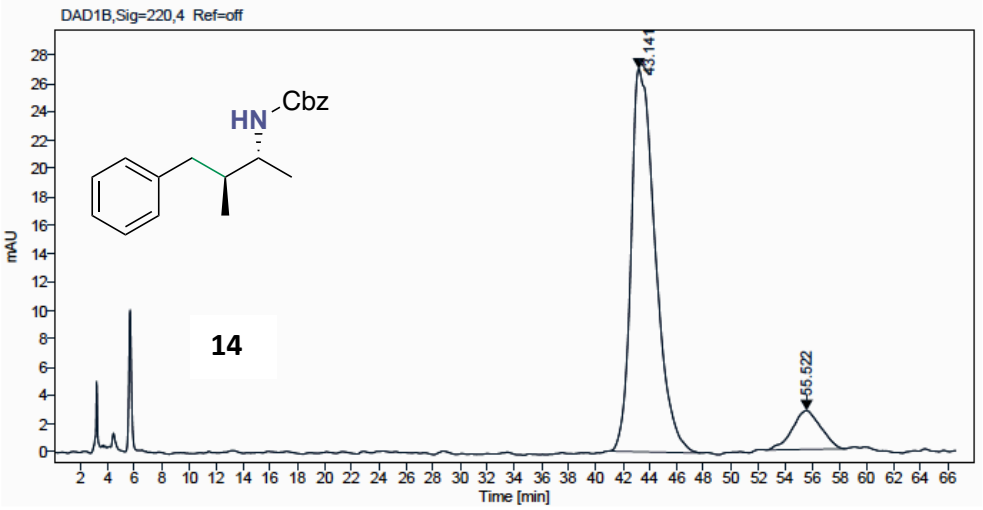
RT [min]	Area	Area%
16.942	214.6432	11.4496
19.936	1660.0353	88.5504
Sum	1874.6786	

For compound **13**, the HPLC analysis revealed an ee of 77% ((88.5504-11.4496)/100). In the top HPLC of the racemic material, the peak at 18.8 min represents two peaks that are a combination of the ratio of the peak at 17.7 and 21.0. In the bottom HPLC assessing ee, only two peaks are present that assumes the second step (the asymmetric ketone reduction with KRED) is ca. 100% ee, hence, the ratio of these two peaks equals the ee of the ERED step.

The dr was determined by NMR spectra. The relative integrations of the two doublet of doublets, corresponding to the protons resulting from the asymmetric ERED-catalyzed reductions, reveal the diastereoisomeric ratio obtained. Moreover, the NMR experiments and the signal ratio is directly proportional to the diastereomeric ratio (dr). Thus, the integration of protons at 2.86 ppm (0.78/1) and 2.80 (0.22/1) (one from each diastereomer) gives a dr of 78/22 (56% de).



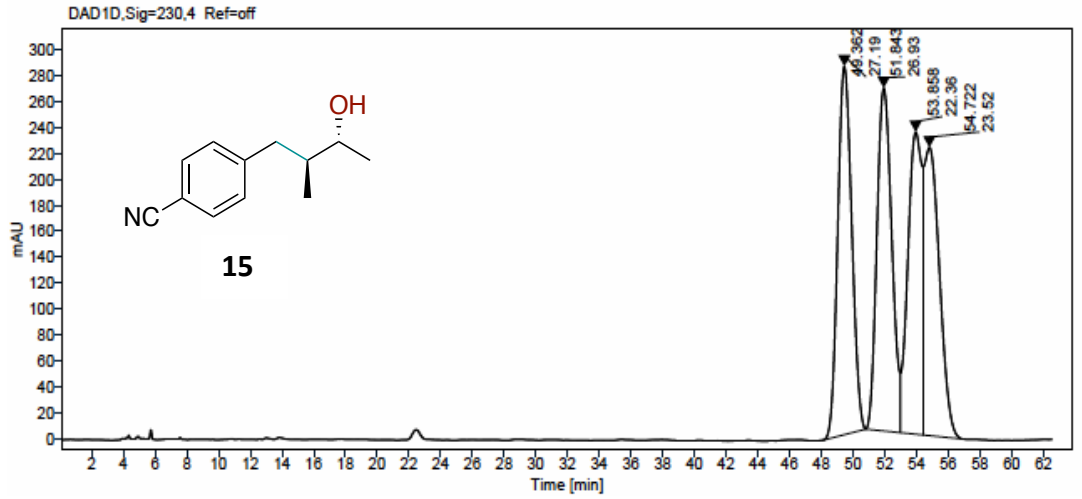
Signal:	DAD1B,Sig=220,4	Ref=off
RT [min]	Area	Area%
33.957	828.3110	24.0537
35.151	901.3046	26.1734
46.186	867.7660	25.1994
58.679	846.2118	24.5735
Sum	3443.5935	



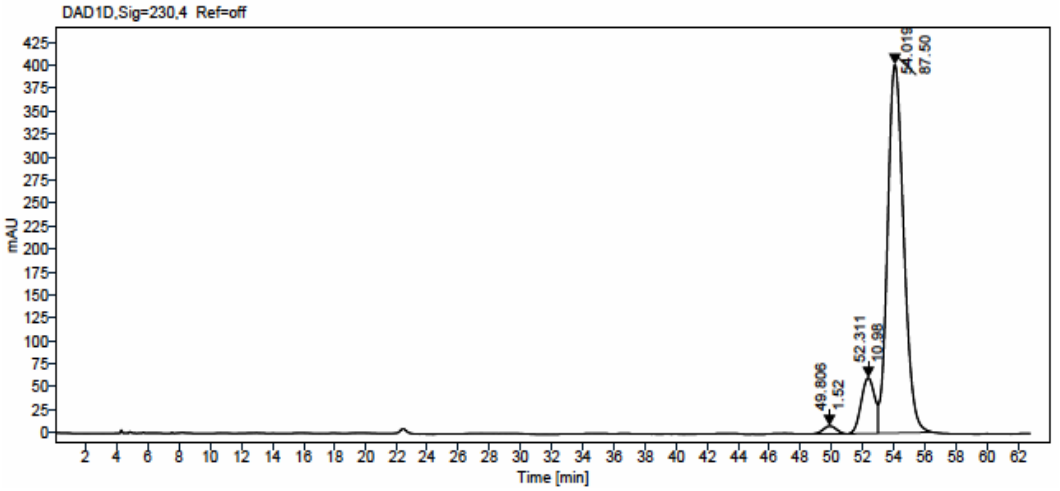
Signal:	DAD1B,Sig=220,4	Ref=off
RT [min]	Area	Area%
43.141	3359.8766	89.3268
55.522	401.4545	10.6732
Sum	3761.3312	

The HPLC analysis reveals an ee of 79% (89.3258-10.6732)/100). The HPLC for racemic material (above) shows 4 peaks of essentially equal area % representing the enantiomers of the two pairs of diastereomers. In the trace below from the reaction sequence, only two peaks are present, and since both outcomes from each enzymatic process is known (as shown), these must represent the ee associated with this particular diastereomer.

The dr was determined by NMR spectra. The relative integrations of the two doublet of doublets, corresponding to the proton resulting from the asymmetric ERED-catalyzed reductions of activated olefins reveal the diastereoisomeric ratio obtained. Moreover, the signal ratio from the NMR experiments is directly proportional to the diastereomeric ratio (dr). So, the integration of protons at 2.80 (0.11 /1) and 2.76 ppm (0.89/1) (one from each diastereomer) enzyme gives a dr of 89/11 (78% de).



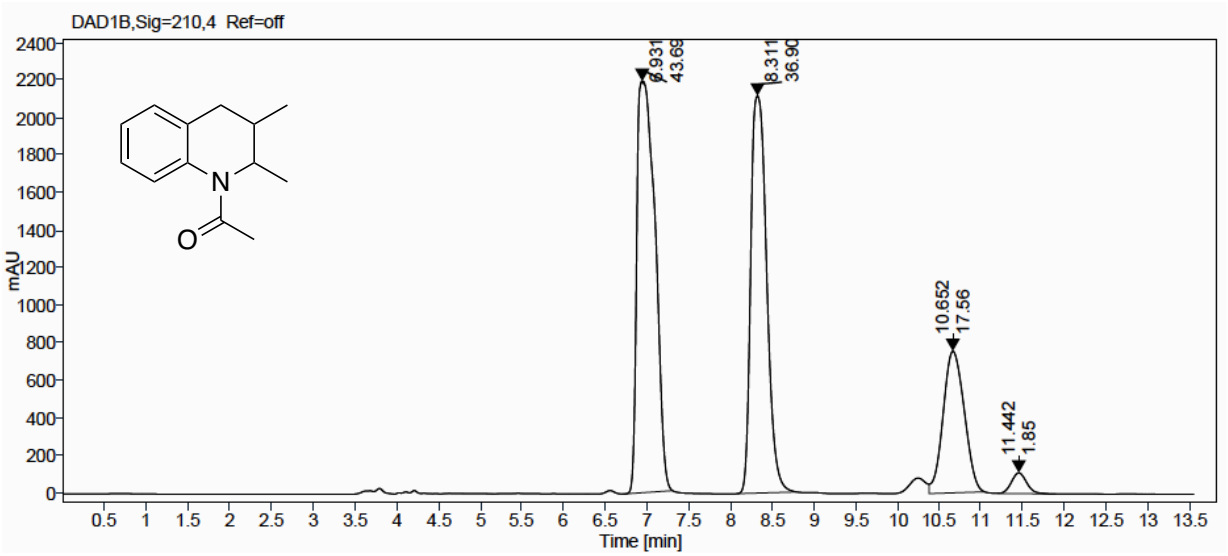
Signal:	DAD1D,Sig=230,4 Ref=off	
RT [min]	Area	Area%
49.362	17387.5329	27.1923
51.843	17218.8645	26.9285
53.858	14296.4819	22.3582
54.722	15040.0540	23.5211
Sum	63942.9332	



Signal:	DAD1D,Sig=230,4 Ref=off	
RT [min]	Area	Area%
49.806	503.8465	1.5218
52.311	3633.9880	10.9756
54.019	28971.7135	87.5026
Sum	33109.5480	

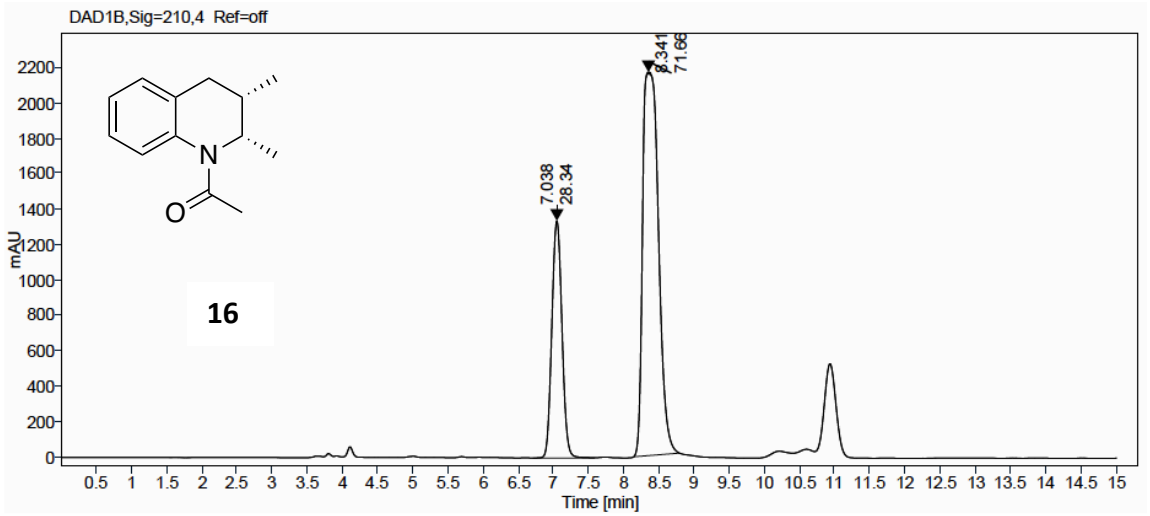
The HPLC data of the racemic material (top HPLC trace) shows both diastereomers in roughly a 1.2:1 ratio. The product (lower HPLC trace) shows three of the four enantiomers of the two possible diastereomers. However, the first two peaks at 49.8 and 52.3 min correspond to one of the two diastereomers, while the largest peak is the only one observed for the major isomer. Hence, the ee is >99%.

Supporting this assignment, the UV spectra, with peaks at 49.81 min and 52.31 min represent enantiomers. The major peak at 54.02 min in the product (lower) HPLC shows no presence of enantiomers. Thus, we can conclude in addition to the ee being >99%, the dr is the ratio between the sum of the first two peaks and the third peak (12:88).



Signal: DAD1B,Sig=210,4 Ref=off

RT [min]	Area	Area%
6.931	33145.5376	43.6868
8.311	27995.2625	36.8986
10.652	13326.6900	17.5650
11.442	1403.3031	1.8496
Sum	75870.7932	



Signal: DAD1B,Sig=210,4 Ref=off

RT [min]	Area	Area%
7.038	13398.9136	28.3395
8.341	33881.0564	71.6605
Sum	47279.9700	

According to the (top) HPLC for the racemic mixture, the peak at 6.93 min has two peaks embedded in this one peak (they are very challenging to separate). The left half of this peak is one enantiomer matched with the peak at 8.31 min since they have the same UV spectra. The right half of the peak at 6.93 min corresponds to the enantiomer which is matched with the peak at 10.65 min. The HPLC data of the product (bottom trace) shows two peaks (the peak at ~11.44 is an impurity). The UV spectra of the peak at 7.04 min and the peak at 8.34 min *are not identical* indicating that each peak represents an enantiomerically pure component of each diastereomeric pair. Thus, with no enantiomeric peaks visible, these HPLC traces indicate that the ee's are >99% for each, while the dr is as shown (28:72).